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STUDIES IN BACTERIOSIS. XIII

A SOFT ROT OF POTATO TUBERS DUE TO *BACILLUS CAROTOVORUS* AND A COMPARISON OF THE CULTURAL, PATHOLOGICAL AND SEROLOGICAL BEHAVIOUR OF VARIOUS ORGANISMS CAUSING SOFT ROTS

BY MARGARET S. LACEY.

(*From the Department of Plant Physiology and Pathology of the Imperial College of Science and Technology, London.*)

So far as can be ascertained there is no previous record of a natural primary infection of potato tubers by *Bacillus carotovorus*, in fact L. R. Jones (7) in his original description of this organism concludes that it was not virulent for the potato. Erwin Smith (10), however, easily obtained artificial infection of both tubers and soft green stems of this plant with strains of *B. carotovorus*.

The present investigation was commenced in March 1924, when a potato badly attacked by soft rot was received, the sender reporting that about 10 per cent. of the bulk of his potatoes were affected in the same way. This potato showed an extensive soft white rot of the tissue inside the vascular ring while the outer tissue was not affected, so that on external examination the potato appeared sound. There was no blackening of the tissue as is usual in tubers attacked by *B. phytophthorus* and the rot was entirely without smell. Microscopic examination of the rotted tissue showed a very heavy mixed bacterial infection, and, as is commonly the case with soft-rot producing organisms, considerable difficulty was experienced in obtaining a pure virulent culture of the causal organism, the isolation plates bearing a large proportion of saprophytic colonies[1].

Finally a pure culture was obtained which, when inoculated into potato tubers, produced a typical rot, that is the whole of the tissue inside the vascular ring was quickly reduced to pulp while the outer

[1] This frequent invasion of rotted tissue by non-pathogenic organisms throws some doubt on the pathogenicity of *Micrococcus prodigiosus*, described by Cavadas (4) as causing a soft rot of the interior of potato tubers. His description of this rot corresponds with the rot just described with the exception that the tissue was stained coral-red by *B. prodigiosus*, which was possibly present as a secondary infection.

tissue either remained sound or was slowly attacked later. The rotted tissue was quite white and without odour.

Morphologically and physiologically the organism causing this rot appeared to be a strain of *B. carotovorus* but in certain characters it approached more nearly the *B. phytophthorus* type. Attention was thus drawn to the very close similarity of the physiological characters of *B. phytophthorus* and *B. carotovorus* and a comparative study of the cultural and pathogenic behaviour of strains of these two species was begun. As there is considerable diversity of opinion whether *B. solanisaprus* should be considered a separate species or a strain of *B. phytophthorus*, a culture of this organism was included.

During the summer of 1924, therefore, experiments were carried out with the following strains:

(1) *B. phytophthorus*, obtained from Dr Appel in 1924.

(2) *B. carotovorus*, strain 50, isolated from rotted violet plants in 1921.

(3) *Strain* 7, the newly isolated strain from internal soft rot of the potato.

(4) *B. solanisaprus*, obtained from Washington through the National Collection of Type Cultures, Lister Institute.

Later, a strongly pathogenic strain of *B. carotovorus* isolated by Dr Bewley from rotted tomato stems, was obtained, and also two cultures from Dr Erwin Smith through the National Collection of Type Cultures.

One of these, indicated in this work as *B. phytophthorus* (E.S.), agreed very closely with the strain from Dr Appel. The other agreed culturally and serologically with the Washington strain of *B. solanisaprus* and is therefore indicated as *B. solanisaprus* (E.S.). Finally, in the spring of 1925 two strains of soft-rotting organisms (25 and 33) were isolated from rotted *Richardia* corms from different sources and various tests were made with these. They differed from *B. aroideae* (Townsend) in producing a little gas in saccharose and glucose broth, but were probably identical with the organism isolated from *Richardia* corms by Bewley (2), which he says was indistinguishable from *B. carotovorus*.

In addition, a few other strains of soft-rotting organisms were included in the serological tests.

Cultural Characters.

Growth on bouillon agar and potato-mush agar slopes was so similar in all the cultures that it was impossible to distinguish between them. On bouillon agar the growth was a semi-transparent streak with slightly

raised and somewhat opaque borders, white by reflected, brownish by transmitted light. Growth on potato-mush agar was very prolific, white at first, later becoming brownish, and with an iridescent appearance.

Bouillon-agar plates (comparison of first four strains). After 24 hours' growth at 24° C. the colonies on all four plates appeared similar, but on magnification, strain 50 was seen to be slightly granular, while the others were hyaline. After 48 hours this granular character had disappeared and the colonies were all alike, semi-transparent, round, smooth, glistening, white by reflected, brownish by transmitted light. After a week the *B. phytophthorus* colonies were somewhat yellower by transmitted light than the *B. carotovorus*, while those of *B. solanisaprus* were somewhat more transparent.

Plates of the two strains 25 and 33 from *Richardia* corms were carefully examined for the radiating surface colonies described by Townsend (11) as characteristic of *B. aroideae*. Strain 25 always produced round colonies with entire edges, of *carotovorus* type. Plates of strain 33 showed a mixture of round and strongly radiating amoeboid colonies. These two types were equally pathogenic on potato and carrot slices and after four transfers through potato slices the radiating type on replating yielded only round colonies. This variability agrees with the findings of Harding and Morse (5) respecting the uncertain nature of this character and its lack of diagnostic value.

Gelatine Stabs (20° C.). Strains 7 and 50 produced rapid infundibuliform liquefaction, commencing in 24–48 hours, liquefaction being practically complete in twelve days. *B. phytophthorus* (Appel) and *B. solanisaprus* (Washington) did not start liquefying until the 6th day, when a slight basin-shaped liquefied area was visible in both. Later, *B. solanisaprus* produced infundibuliform liquefaction, while that of *B. phytophthorus* was stratiform. Liquefaction in both was complete after two months. After three weeks a strong brown colour was produced in the lower half of the *phytophthorus* culture. Later tests showed that strains 25 and 33 agreed with *B. carotovorus* in producing rapid infundibuliform liquefaction. *B. phytophthorus* (E.S.) produced stratiform liquefaction starting on the 4th day, and *B. solanisaprus* (E.S.) infundibuliform liquefaction starting on the 3rd day. Jennison (6) obtained more rapid liquefaction of *B. atrosepticus* strains than was here obtained for *B. phytophthorus*.

Production of Indol. No indol was produced in any of the cultures even after six weeks' incubation, although a positive indol reaction was obtained in a control culture of *B. campestre* tested under similar

conditions. Harding and Morse(5) obtained a slight indol reaction in all their *carotovorus* strains, but state that on various occasions the red colour produced by the test was not sufficient to be considered positive.

The negative test in the *phytophthorus* cultures agrees with Jennison's(6) findings on all his strains of *B. atrosepticus* under which he includes *B. phytophthorus* (Appel), *B. solanisaprus* (Harrison) and *B. melanogenes* (Pethybridge and Murphy).

Table I.

Comparison of Various Reactions.

	B. phytophthorus (Appel)	*B. solanisaprus* (Washington)	Strain 7	*B. carotovorus*
Optimum temp. for growth	30° C.	25–30° C.	25–30° C.	25° C.
Growth at 37° C.	None	Very slight	Slight	Slight
Thermal death point	47° C.	50° C.	48° C.	50° C.
Diastatic activity	None	Very slight	Strong	Strong
Reduction of nitrate	+	+	+	+
MacConkey's medium	White growth, medium turned orange	Like *phytophthorus*	Like *phytophthorus*	Like *phytophthorus*
		(*B. coli* gave a red growth and the medium turned red)		
Fermi's solution	24 hrs., faintly turbid. 48 hrs., fluid clear, bacteria appear agglutinated on the sides and bottom of the tube	Strong turbidity after 48 hrs., no clearing	Like *solanisaprus*	Like *solanisaprus*
				B. carotovorus 50
Uschinsky's solution plus litmus	Very slight growth, fluid remained clear, with a gradually increasing deposit and a heavy pellicle breaking up into pseudozoogloea. A slight white ring was formed. No colour change but the litmus was gradually reduced until the fluid became colourless	Good growth, litmus slightly blued, later reduced. After 1 month fluid became yellowish and very turbid with thick white ring and mucoid deposit	Good growth, litmus slightly reddened, then rapidly reduced. Slight white ring. After 1 month fluid was nearly clear and colourless with a thick mucoid deposit	Good growth, litmus slightly reddened, no reduction. Heavy white ring, only slight deposit after 1 month
Litmus milk	Slow production of acid. Coag. of casein delayed in 1st test till 11th, in 2nd till 16th day. Firm clot then formed. No digestion after 3 months. No reduction of litmus	Acid produced. Firm clot formed by 4th day, followed by digestion, which was complete in 6 weeks. Litmus reduced	Acid produced. Firm clot formed by 4th day. Possibly very slight digestion, but clot still present after 3 months. Litmus reduced at first, later pink colour returned	Acid produced. Clotting started on the 4th day. No digestion after 3 months. Litmus reduced, later pink colour returned

As regards the other strains, *B. phytophthorus* (E.S.) and *B. solanisaprus* (E.S.) gave the same reactions in Fermi's and Uschinsky's solution as *B. phytophthorus* (Appel) and *B. solanisaprus* (Washington) respectively.

In milk, however, *B. phytophthorus* (E.S.) gave digestion of the casein, thus agreeing with Jennison's *B. atrosepticus* strains, while in

B. solanisaprus (E.S.) cultures clotting was delayed for three weeks and there was no subsequent digestion and no reduction of litmus, therefore agreeing in this respect with *B. phytophthorus* (Appel). *B. carotovorus* (Bewley) agreed with strain 7's behaviour in Uschinsky and litmus milk while strains 25 and 23 gave the same reaction as *B. solanisaprus* (Washington) in these media.

Fermentation of Sugars. A 1 per cent. solution of each sugar in peptone water, to which litmus solution was added, was used for these tests. Acid was produced in all strains in glucose, lactose, saccharose and mannite. Gas production was variable in amount, the two *phytophthorus* strains only producing very small gas bubbles or occasionally none at all. Thus in two tests with *B. phytophthorus* (Appel) there was no gas production in glucose, and only a very small gas bubble in one tube of mannite, the second failing to give gas; while *B. phytophthorus* (E.S.) gave only a very minute gas bubble in lactose after three days. *B. solanisaprus* (Washington) produced some gas in all, while *B. solanisaprus* (E.S.) failed to give gas in two tests in glucose. Strain 7, *B. carotovorus* (50) and *B. carotovorus* (Bewley) produced gas in all, *B. carotovorus* (50) producing the least and *B. carotovorus* (Bewley) the most. The two strains 25 and 33 from *Richardia* corms produced a small amount of gas in glucose and saccharose, and strain 33 in mannite, while neither produced any gas in lactose.

Maltose peptone water. This medium clearly distinguishes the *phytophthorus* from the other soft-rotting strains, for all strains of *B. phytophthorus* tested produced acid and gas from maltose, while the *carotovorus*, strain 7, *solanisaprus* 25 and 33 strains gave neither acid nor gas.

Reversal of reaction after some days' growth in carbohydrate media. Wolf and Foster (12), studying the fermentable activity of some plant bacteria, state that *B. carotovorus* will reach its final acid reaction in three days, and after that, in broths with an initial sugar content of 0·1 per cent., there is a reversal of reaction and a rise of *p*H said by Ayers and Ruff (1918) to be due not to formation of ammonia but to fermentation of organic acids to carbonates. In media containing a higher percentage of sugar there was no such reversal.

The five strains *phytophthorus* (Appel), *solanisaprus* (Washington), strain 7, *carotovorus* (50) and *carotovorus* (Bewley) were tested for this reversal. Tubes of peptone water containing 1, 0·3 and 0·1 per cent. of glucose, lactose or saccharose respectively were inoculated with a drop of a 24 hour broth culture and incubated at 20° C. At intervals over a period of 16 days, small quantities were withdrawn by a sterile pipette

and the *p*H value tested. Repeat tests were made of the *solanisaprus* lactose solutions, and also of the 0·3 per cent. glucose and saccharose.

Table II gives the results obtained, the presence or absence of reversal being indicated by a + or − sign respectively and the highest *p*H value reached being shown in the first columns.

Table II.

Sugar %		Initial *p*H value	*Phytophthorus* Highest *p*H value	*Phytophthorus* Reversal	*Solanisaprus* Highest *p*H value	*Solanisaprus* Reversal	*Carotovorus* 50 Highest *p*H value	*Carotovorus* 50 Reversal	Strain 7 Highest *p*H value	Strain 7 Reversal	*Carotovorus* Bewley Highest *p*H value	*Carotovorus* Bewley Reversal
Glucose	1	7·0	4·8	−	4·8	−	4·8	−	4·8	−	4·8	−
,,	0·3	7·4	4·8	−	5·4	+	4·8	−	4·8	−	4·8	−
,,	0·1	7·6	4·8	+	5·6	+	5·1	+	5·0	+	4·9	+
Lactose	1	7·3	4·8	−	5·0	−	4·8	−	4·8	−	4·8	−
,,	0·3	7·8	4·8	−	5·0	−	4·8	−	4·8	−	4·8	−
,,	0·1	7·5	5·2	+	5·3	−	5·0	+	5·0	+	4·9	+
Saccharose	1	7·3	4·8	−	4·8	−	4·8	−	4·8	−	4·8	−
,,	0·3	7·8	4·8	−	5·8	+	4·8	−	4·8	−	4·8	−
,,	0·1	7·4	4·8	+	5·4	+	4·8	+	5·0	+	4·9	+

The three *B. carotovorus* strains therefore agree with that of Wolf and Foster(12) in producing a reversal of reaction in the 0·1 per cent. sugar peptone water, and *B. phytophthorus* behaves in a similar manner. *B. solanisaprus*, on the other hand, produces a reversal in the 0·3 per cent. as well as the 0·1 per cent. glucose and saccharose solutions, but produces no reversal at all in any of the lactose solutions. This is probably correlated with the length of time taken to reach the highest acidity. Whereas in the 0·3 and 0·1 per cent. glucose and saccharose broths the highest *p*H value is reached in 2–4 days, in the *solanisaprus* lactose cultures it is not reached until the 11th day, when growth has practically ceased.

Lactose is less readily fermented by all these strains than glucose or saccharose.

The macroscopic appearance of *B. phytophthorus* in saccharose broth is very characteristic. A slight turbidity is produced at first but the bacteria soon fall to the bottom of the tube as if agglutinated, so that the supernatant fluid is perfectly clear, often after only 48 hours' growth. Growth continues at the bottom and a thick deposit is gradually accumulated.

Pathological Characters.

Inoculation of potato slices. Numerous tests were carried out with all strains on the character of the rot produced on thick potato slices in deep petri dishes lined with moist blotting paper kept at various tem-

peratures from 15–25° C. The rot in each case was always of the same type whether "new" or "old" potatoes were used, and the results can be summarised as follows:

Rot produced by *B. phytophthorus* (strains Appel and E.S.). Equal rotting of all tissue both inside and outside the vascular ring. Heavy jet black border produced round the rotted tissue, which was white at first but turned pinkish on exposure to light. Occasionally a strong fishy smell was produced, but this was variable and seemed to depend on the variety of the potato used.

B. solanisaprus (Washington and E.S.). Not very vigorous. Much exudation of clear fluid. Rot white at first, later often rather brownish. Soon dried out. No black border ever produced.

B. carotovorus (strains 7, 50, Bewley) and strains 25 and 33. Tissue inside the vascular ring much more easily and completely attacked than that outside, the former often being completely rotted through while the cortex was still unattacked. Pure white rot, no blackening and no smell.

The *p*H values of the various rots were taken on several occasions in an attempt to confirm Paine and Chaudhuri's (9) finding that *B. phytophthorus* produced an alkaline rot while that of *B. solanisaprus* was acid[1]. In these tests, however, the rots were alkaline with all the strains, and at whatever time of the year the experiments were made. It was thought that the fermentation of the sugar present in new potatoes might have an influence on the final *p*H value, but this was found not to be the case. Thus in one test the initial *p*H of the potato being about 6·0, the following values for the rotted tissue were obtained on the third day: *B. phytophthorus* 8·0, *B. solanisaprus* 7·6, *B. carotovorus* (various strains) 7·8–8·4.

Carrot slices. All *B. carotovorus* strains and strains 25 and 33 produced a very vigorous rot. *B. solanisaprus* (E.S.) was also vigorous; the rot was accompanied by much exudation of fluid.

B. phytophthorus (E.S.) produced only a very slight rot (2 mm. deep after four days at 20° C.), while with *B. phytophthorus* (Appel) and *B. solanisaprus* (Washington) all tests were negative, both with new and old carrots. (Dr Berridge (1), at a rather higher temperature obtained some rotting with these two strains.)

[1] I am entirely unable to account for the discrepancy between these results and those of Miss Lacey. The results given in the above cited paper have never been obtained since, and unfortunately the strain of *B. solanisaprus* with which Mr Chaudhuri worked is no longer available. S. G. Paine.

Table III.

Plant Inoculations.

Plant inoculated and method	*B. phytophthorus* (Appel)	*B. solanisaprus* (Washington)	Strain 7	*B. carotovorus*
Young potato shoots, cut ends placed in water containing pathogen. 2 shoots for each experiment	3 *days*. Stems blackened for 1 in. with black streaks above. Leaves dead. 4 *days*. Dead. Big gas bubbles under the epidermis	One remained healthy. 2nd, after 4 days about 1 in. of stem rotted, but remained green. Gas bubbles under the epidermis	3 *days*. 3–4 in. of stem rotted, but green. 4 *days*. Vascular bundles showed through the rotted tissue as brown streaks	—
Potato plants in ground stem inoculated about 1 in. above soil with hypodermic injection	Produced typical "Blackleg," 18 days. One examined, pith of stem rotted to junction with root. Rotted tissue black with a slight fishy smell. Vascular tissue above the inoculated part unaltered in colour. No splitting open of stems	Negative result from 6 inoculations	No blackening of tissue. Stems split open round the inoculated points for 1–2 in. showing the entire destruction of the pith. Stems pulled up showed rotting of the pith to root and above the inoculation the vessels were browned for 4 in.	Like strain 7 but not so vigorous
				B. carotovorus 50
Potato tubers, plug cut out, end inoculated and plug replaced. Kept at room temperature. 2 potatoes each	6 *days*. Strong blackening visible through the epidermis over the half surrounding the plug. This half completely rotted to the epidermis, white with a deep black border, turning pink on exposure to light. 24 hours after cutting rotted tissue was black and there was a strong fishy smell	One negative, 2nd good white rot over whole interior, cortex sound. Rot surrounded by a brown corky area. No blackening and no smell, rot remained white	White rot for some distance round inoculation point, then cork formed and rot dried out	Both negative
Potato tubers at 20° C. Plug inoculation. 2 each	As at room temperature. 14 *days*. Outer layers of one were completely rotted, leaving a hard plug in the centre. 2nd only half rotted	As at room temperature. Rotted tissue brownish	7 *days*. (1) Pith reduced to pulp, cortex rotted but still holding together. (2) Whole interior a soft mush, cortex still sound in places. Pure white	Like strain 7, but rather slower development
Potato tubers at 20° C. Prick inoculation. One each	9 *days*. Good rot, with jet black border showing through the epidermis	Negative	Whole potato, with the exception of part of the cortex, reduced to a soft white pulp	Negative
Young carrot roots. Prick inoculation into stem end. At room temperature. 3 for each	All negative	All negative	Pith rotted for same distance down from the inoculated point	Like strain 7
Turnip roots. Prick inoculation at stem end at 20° C.	Slight rotting and blackening of the cortex only. Stem unattacked	About 1½ in. of the pith rotted. *Stem* completely rotted	1 cm. of pith rotted, pure white. *Stem* appears sound but whole of pith is rotted	Slight rotting round the pith only
Turnip slices	White rot with black border. Unpleasant smell	Slight brownish rot, no smell	Strong rot of pith only, rather brownish, no smell	—
Celery shoots, prick inoculation	Negative result	All young inside leaves rotted, outer older leaves unattacked	Rotted round the pricks, but dried out	Heavy rot of young inside leaves which spread until whole shoot was rotten

Inoculation of Vicia faba *plants.* *B. carotovorus* (Bewley), strain 7, and *B. phytophthorus* (E.S.) all attacked the stems of bean plants which turned black round the inoculated area, the part above falling over. The other strains gave negative results.

Numerous other inoculations were done with the first four strains only and are summarised in Table III.

From these inoculations it is evident that the rot caused by *B. phytophthorus* can always be distinguished from the others by the strong black colour produced round the rotted tissue. Also while *B. phytophthorus* can easily attack the outer layers of tubers or roots, *B. carotovorus* species quickly destroy the pith, but only slowly attack the cortical tissue.

The rot produced by *B. solanisaprus* is more watery than *carotovorus* and usually somewhat brownish in colour, whereas *B. carotovorus* rots are quite white.

Serum Agglutinations.

The sera used for these tests were prepared by Dr Brooks at the Lister Institute, to whom the author wishes to express her thanks. The cultures used for the preparation of the *carotovorus* and *phytophthorus* sera respectively were *B. carotovorus* (strain Bewley) and *B. phytophthorus* (Appel). The sera were of a very high titre.

Considerable variability in the degree of agglutination was experienced in various tests with the same organisms[1], although the agglutination only differed in amount and not in kind, and it was found that the acidity or alkalinity of the water used for diluting the serum and for making bacterial emulsions considerably influenced the result.

Finally, a buffered physiologic salt solution recommended by Mason and Sanford (8) for serological use was tried and found to give very clear cut results. Table IV shows the amount of agglutinations obtained by this method after four hours' incubation at 37° C.

Table V shows the maximum agglutination obtained with the sera for a few other strains of soft-rotting organisms.

These tables show that while there is a close relationship between *B. carotovorus* and *B. phytophthorus* species, there are yet clearly marked specific differences. Strains 25 and 33 belong to the *carotovorus* group, while *B. solanisaprus* (Washington) shows a slight group agglutination for both *B. phytophthorus* and *B. carotovorus* sera, which is, however, slightly stronger for the latter. *B. solanisaprus* (E.S.) failed to give any

[1] Dr Brooks also found great variations in agglutinations with these organisms.

Table IV.

Culture	Serum	Serum dilutions 1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	Control
B. phytophthorus Strain Appel	*Carotovorus*	++	++	−	−	−	.	.	.	−
	Phytophthorus	++	+++	+++	+++	++	+	+	−	.
B. phytophthorus Strain E.S.	*Carotovorus*	++	+	−	−	−	.	.	.	−
	Phytophthorus	+	++	++	++	++	++	−	−	−
B. carotovorus (Bewley)	*Carotovorus*	+++	+++	+++	++	++	++	+	−	.*
	Phytophthorus	+++	+++	+++	++	++	+	.	−	−
B. carotovorus Strain 50	*Carotovorus*	+		−		.	.	.	.	−
	Phytophthorus	−	−	.	.	.	.	.	.	.
Strain 7	*Carotovorus*	++	++	++	++	−	−	−	.	−
	Phytophthorus	+		.	.	−		−	.	.
B. solanisaprus (Washington)	*Carotovorus*	±	−	.	.	.	.	.	.	−
	Phytophthorus	±	.	−	−	.	.	.	.	.
B. solanisaprus Strain E.S.	*Carotovorus*	.				.	.	.	.	−
	Phytophthorus	−	−	−		.	.	.	.	.

* *Carotovorus* (strain Bewley) was slightly self-agglutinable, and control tubes always showed some agglutination if left for more than 4 hours. The specific nature of this agglutination is therefore doubtful.

Table V.

Culture	Source	*Carotovorus* serum	*Phytophthorus* serum
Strain 25	*Richardia* corm	1/1000	—
„ 33	„	1/100	—
„ 17 *a*	Potato lesions	—	1/200
„ X	Rotted celery	—	1/1000

agglutination with these two sera, but Dr Brooks (3) has proved its agglutinability with *B. solanisaprus* (Washington) serum.

Conclusion.

Cultural, pathological and serological tests all show that a close relationship exists between the three species *B. carotovorus*, *B. solanisaprus* and *B. phytophthorus*, but there are nevertheless sufficiently marked and constant differences to warrant their continued separation into different species.

B. phytophthorus can be distinguished culturally from *B. carotovorus* or *B. solanisaprus* by:

(1) Its production of acid and gas in maltose broth.

(2) Behaviour in Uschinsky's solution.

(3) Rapid clearing in Fermi's solution and in saccharose broth.

(4) Absence of diastatic action.

(5) Failure to grow at 37° C.

As regards the pathogenic behaviour, the constant jet black appearance of tissues attacked by *B. phytophthorus* easily distinguishes this from a *B. carotovorus* or *B. solanisaprus* infection. These two latter

appear more nearly allied, but can be distinguished culturally by their behaviour in Uschinsky's and Fermi's solutions and by the acid and alkali production in sugar peptone water. Pathogenically, the rot caused by *B. solanisaprus* is more watery, and the tissues more browned than a *B. carotovorus* rot. Lastly, although there is a common group agglutinin, the species can be separated by serum agglutinations, which agree with Dr Berridge's (1) chemical agglutinations in the grouping of the different strains tested.

In conclusion, I wish to express my thanks to Prof. S. G. Paine for his advice and criticism given throughout the course of this investigation.

SUMMARY.

An internal soft rot of potato tubers caused by *B. carotovorus* is described. Various strains of *B. phytophthorus*, *B. solanisaprus* and *B. carotovorus* are compared with respect to their cultural, pathological and serological behaviour, and the differences seem sufficient to warrant their continued separation into distinct species.

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STUDIES IN BACTERIOSIS. XIV

CHEMICAL AGGLUTINATION AS A MEANS OF DIFFERENTIATING BACTERIAL SPECIES CAUSING SOFT ROT OF POTATOES AND OTHER VEGETABLES

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Jennison in a recent paper(6) maintains that the various species of bacteria which cause blackleg and soft rot of potato, *i.e.*, *B. atrosepticus* (van Hall), *B. solanisaprus* (Harrison), *B. melanogenes* (Pethybridge and Murphy) and *B. phytophthorus* (Appel), are all variants of one species which he proposes should be called *B. atrosepticus*. Morse(9) also considers that the variable cultural characteristics which distinguish the three first arise merely from variations in conditions of culture of the strains. With regard to *B. phytophthorus* (Appel), Dr Erwin Smith(10) found that it differed from the other species in various details, and regards it as very closely related but not identical with *B. solanisaprus*.

Having already found that the maximum acid agglutination points for *B. solanisaprus*, *B. phytophthorus* and *B. carotovorus* differed slightly but quite definitely from each other(2), the behaviour of these three species in agglutinating solutions was further examined; certain of these reactions seem to furnish a ready means of distinguishing and identifying them.

The strain of *B. solanisaprus* used was obtained from the National Collection of Type Cultures at the Lister Institute and was originally given by Dr Erwin Smith; the *B. phytophthorus* strain was obtained from Dr Appel in 1924 while the *B. carotovorus* used as the type strain was one isolated from tomatoes by Dr Bewley at the Cheshunt Experimental Station. Three other strains isolated in this laboratory, one from rotting violet stems (No. 50) and two from potato (No. 7 and E.B.), were all proved to be *carotovorus*, both by cultural tests and by serum and chemical agglutination tests(5). All these six strains rotted slices of raw potato and carrot, the rot commencing in one day after inoculation, at a temperature of 21° C. The *B. carotovorus* (Bewley) and

B. phytophthorus cultures were the most vigorous on both, while *B. solanisaprus* and *B. carotovorus* (No. 50) spread more slowly in the tissue. Subsequently two other strains of *B. phytophthorus*, one of *B. solanisaprus* and one isolated from rotting *Richardia* rhizomes were also subjected to the same chemical agglutination tests, for purposes of comparison with the results of agglutination tests with immune serum carried out by Miss Lacey in this laboratory (8).

Acid Agglutination Tests.

In a series of agglutination tests in which Sørensen's citric acid buffer solution was employed, the maximum point for *B. solanisaprus* was found to be about *p*H 2·0 and for *B. carotovorus* the maximum was near *p*H 3·0 (2).

Using mixtures of *N*/10 acetic acid and *N*/50 NaOH, *B. solanisaprus* gave maximum agglutination at *p*H 2·4, two *B. carotovorus* strains at *p*H 3·5–3·7 while for *B. phytophthorus* the maximum point was about *p*H 3·2.

The differences in the results with the two acids, citric and acetic, is probably due to the difference in valency; various workers (Bechhold (1), Buxton and Shaffer (4), Eisenberg (5)) have shown that the higher the valency the greater the H-ion concentration required for the agglutination of any given species of bacterium.

These maximum values lie too close together to be easily determined; by using certain acids alone, however, progressively diluted with distilled water, the differences in agglutinability become very striking.

In the first series tannic acid was used; since sensitiveness to this acid is of interest as bearing on the virulence of these species of bacteria towards tannin-containing plants and organs.

Table I gives the reactions of 24-hour bouillon agar cultures of the three species to successive dilutions of a 6 per cent. tannic acid solution after incubation for three hours at 30° C. These tests were made in open Max Neisser dishes as the presence of air seemed to favour the agglutination, but similar differences in agglutinability are evident when the ordinary Dreyer tubes are used, though in this case agglutination ceases at a lower dilution.

B. carotovorus is evidently extremely sensitive to tannic acid; the emulsion is dead after four hours in dilutions of 3/16 per cent. and below, and a three-day-old culture was agglutinated in a dilution of 3/1024 per cent. The *p*H value of the 3 per cent. solution was approximately 3·0,

Table I.

Showing Agglutination in Tannic Acid Solution.

Total dilution %	*B. solanisaprus*	*B. phytophthorus*	*B. carotovorus*	
3	–	+ + +	+ + +	Immediate
3/2	–	+ +	+ + +	Immediate
3/4	–	+ +	+ + +	Immediate
3/8	–	+ + Partial	+ + +	Immediate
3/16	–	Slight	+ + +	
3/32	–	„	+ + +	
3/64	–	–	+ + +	
3/128	–	–	+ + Partial	
3/256	–	–	+ Slight	

hence *B. carotovorus* gives with tannic acid a maximum agglutination point similar to that found with citric acid.

A similar series of tests was made with progressive dilutions of $N/10$ acetic acid. *B. phytophthorus* was strongly agglutinated in all dilutions up to $N/80$ since the H-ion concentration of the acid (pH 2·8) lies near the optimum agglutination point for this species, while *B. solanisaprus* and *B. carotovorus* whose agglutination maxima lie respectively above and below this point react only slightly or not at all. *B. carotovorus* was found to be killed by the acid in all dilutions down to $N/320$ within one hour, the incubation temperature being 30° C.

Agglutination with Sulphates.

The three soft rot organisms also showed marked differences in their agglutinability by solutions of sulphates. Ammonium sulphate was chiefly used in dilutions of 50, 20 and 10 per cent. in distilled water. These solutions were further diluted in the agglutination tubes in the proportions of sulphate solution to water 10 : 0, 9 : 1, 8 : 2 etc., and five parts of emulsion of the organism to be tested were added. The emulsions were not washed or treated in any way since such treatment appears to remove specific differences of agglutinability; Küster, Lange and Potthoff(7) have shown that strongly agglutinating strains of the coliform group lost all agglutinability after one washing with distilled water in the centrifuge, and that washing with salt solution and serum often had the same effect.

The degree of agglutination was noted after two hours' incubation at 30° and again after four hours' incubation and finally after standing at room temperature overnight. They were recorded as follows:

+ + + (complete agglutination).
+ + g.
+ + (flocculation visible to the naked eye).
+ g.
+ clearly visible with × 10 magnification.
+ sl. (slight).

It is only necessary to give one full series of results as an example. The strain used was *B. carotovorus* (No. 50). The percentage dilutions, including dilution with the emulsion, is given to the nearest whole number.

Table II.

50 % Am_2SO_4 drops	H_2O drops	Emulsion drops	Dilution %	Agglutination	
				After 2 hrs.	After 4 hrs.
10	0	5	33	+ + +	+ + +
8	2	5	27	+ + +	+ + +
6	4	5	20	+ g.	+ g.
5	5	5	17	+ sl.	+ g.
4	6	5	13	–	+
3	7	5	10	–	+ sl.
2	8	5	7	–	–
1	9	5	3	–	–

In a series of such tests it was found that whereas *B. solanisaprus* was inagglutinable in dilutions of 10 per cent. and above, all strains of *B. carotovorus* were agglutinated in dilutions ranging from 10–3 per cent. but not by a 1 per cent. dilution, while *B. phytophthorus* only reached its limit of agglutination in a dilution of ·8 per cent.

The tests were repeated with solutions of sodium and magnesium sulphate equimolecular with 20 per cent. ammonium sulphate; the agglutinating effect was practically the same in all three cases.

The agglutinability of emulsions from rotted potato was also tested; in some cases it was slightly greater than that of emulsions from bouillon agar but in every case *B. solanisaprus* was markedly the least agglutinable species, and *B. phytophthorus* the most sensitive to these solutions (Table III). *B. carotovorus* (No. 50) was however in all tests less agglutinable than the other *B. carotovorus* strains, showing finer flocculation and a lower limit, thus approaching *B. solanisaprus* in character.

Since Morse (9) emphasises the importance of subculturing these types repeatedly before making comparative tests of their reactions, so that they are all in a thoroughly vigorous condition of growth, the *B. solanisaprus* and *B. phytophthorus* strains as well as *B. carotovorus* (Bewley)

were subcultured in "Jardox" peptone broth for nine days in succession and again tested with ammonium sulphate. Although their sensitiveness to the solutions had slightly increased in all cases, their relative agglutinability was the same as before, differentiating *B. solanisaprus* very strikingly from *B. phytophthorus* (Table III).

Table III.

Agglutination with Sulphates.

		B. solanisaprus		*B. carotovorus* (Bewley)		*B. carotovorus* (No. 50)		*B. carotovorus* (E.B.)		*B. carotovorus* (No. 7)		*B. phytophthorus* (Appel)	
		2 hrs.	4 hrs.	2 hrs.	4 hrs.	2 hrs.	4 hrs.	2 hrs.	4 hrs.	2 hrs.	4 hrs.	2 hrs.	4 hrs.
$(NH_4)_2SO_4$	20 %	+g.	++	+++	+++	+g.	++g.	++g.	+++	++	++g.	++g.	+++
	10 ,,	—	—	+g.	++g.	—	+sl.	+sl.	++g.	++	++g.	++	++g.
	3 ,,	—	—	—	—	—	—	—	—	—	—	+g.	+ r
	1 ,,	—	—	—	—	—	—	—	—	—	—	+sl.	+
Na_2SO_4	*1·5 *N*	—	—	+	++	++g.	++g.	++g.	+++	+	+g.	+	++g.
	·45 ,,	—	—	—	+	+sl.	+	+	++	—	+sl.	+g.	++g.
	·15 ,,	—	—	—	—	—	—	—	—	—	—	+sl.	+g.
$MgSO_4$	1·5 ,,	—	—	++	+++	++g.	++g.	++	+++	+	+g.	+	++g.
	·45 ,,	—	—	+sl.	+	—	+	+	++	—	—	+g.	++g.
	·15 ,,	—	—	—	—	—	—	—	—	—	—	+	+g.
Potato cultures, 2 days old	20 %	+sl.	+sl.	+g.	+++	+sl.	+	+g.	+g.	+g.	+g.	+	+ r
	10 ,,	—	—	+	+++	—	—	+g.	+g.	+	+g.	+g.	++g.
	3 ,,	—	—	—	+sl.	—	—	+	+	+	+	++	++g.
	1 ,,	—	—	—	—	—	—	—	—	—	—	+g.	+ r
Bouillon agar after 9 transfers	20 ,,	++	+++	+++	+++							++g.	+++
	10 ,,	+g.	++g.	+	+++							++	+++
	7 ,,	—	—	+	++							+g.	+++
	3 ,,	—	—	—	—							+	++
	1 ,,	—	—	—	—							+	+g.

* 1·5 *N* Na_2SO_4 is approx. equimolecular with 10 % $(NH_4)_2SO_4$.

Comparison between the serological and chemical agglutination reactions of soft-rot organisms.

The six strains used in the preceding experiments had all been tested serologically as well as culturally by Miss Lacey with sera prepared from the *B. carotovorus* (Bewley) and *B. phytophthorus* (Appel) types. The results of the acid agglutinations agreed in every case with those obtained with serum.

Three additional strains were also tested and the results compared.

1. *Strain* 25. Isolated from rotting rhizomes of Arum Lily.

This agglutinated immediately with 3 per cent. tannic acid and in four hours in dilutions up to $\frac{3}{64}$ per cent. With acetic acid there was only a trace of agglutination in $N/2$–$N/20$, but none in the higher dilutions.

With ammonium sulphate there was distinct agglutination up to 5 per cent. and a trace in a 4 per cent. solution, but none in 3 per cent. These reactions confirm the view that this strain is practically identical with *B. carotovorus*.

2. Two cultures contributed by Dr Erwin Smith; one labelled *B. phytophthorus* and the other *B. carotovorus* were obtained from the National Collection of Type Cultures, Lister Institute. *B. phytophthorus* gave agglutinations typical of this species, *i.e.*, up to $\frac{3}{8}$ per cent. with tannic acid and partially up to $\frac{3}{32}$ per cent. in four hours up to $N/40$ with a trace in $N/80$ with acetic acid, and definite agglutination in $1\frac{2}{3}$ per cent. with ammonium sulphate. These reactions correspond almost exactly with those of *B. phytophthorus* (Appel).

3. The other culture, labelled *B. carotovorus*, however, only agglutinated up to 13 per cent. with ammonium sulphate and in the acid solutions behaved more like *B. solanisaprus*. It was therefore replated on gelatine and the liquefying colonies after subculturing gave all the agglutination reactions typical of *B. solanisaprus* (Washington). This result confirmed Miss Lacey's determination of the species by serum agglutination and agrees with the relationship given by Brooks, Nain and Rhodes, who found that this culture does not agglutinate with immune serum prepared from *B. carotovorus* (Bewley), but on the other hand gives a serum which agglutinates *B. solanisaprus* (Washington).

CONCLUSION.

Chemical agglutination tests, which appear to be as reliable as serum agglutination tests within the limits of this well-defined group of bacteria, show that *B. solanisaprus* and *B. phytophthorus* are not identical organisms. Both are closely related to *B. carotovorus*, which, in these reactions shows itself intermediate between the two.

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THE VIABILITY OF THE WINTER SPORANGIUM OF *SYNCHYTRIUM ENDOBIOTICUM* (SCHILB.) PERC., THE ORGANISM CAUSING WART DISEASE IN POTATO

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(With 1 Diagram.)

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I. A STAINING METHOD FOR TESTING THE VIABILITY OF THE WINTER SPORANGIUM.

Introduction.

In attempting to eradicate *Synchytrium endobioticum* from contaminated soil the most rational and economical sequence of study is to determine what conditions bring about death of the causal organisms *in vitro* and to reproduce these conditions first on a pot scale and afterwards in the field. This systematic method of attack has not been possible because no technique was known for testing *in vitro* the viability of the organism and because methods of pot experiment proved unsatisfactory. Field experiments though slow and costly have therefore been used to a large

extent independently of work in the laboratory and glasshouse. Extensive field tests have been carried out by a number of workers including Malthouse (7), Schaffnit and Voss (10) and members of this institution (9), but no satisfactory and economic means of sterilising soil contaminated with *Synchytrium endobioticum* has been found. Further, field results are difficult to interpret in that plots treated in the same way often give different results in different years. Precise methods are therefore needed so that fundamental knowledge regarding the viability of the organism causing Wart Disease may be obtained.

Pot experiments have been used to a limited extent in the study of Wart Disease, but were found unreliable in that infection even of the controls appeared erratic. A study was therefore made of the factors controlling infection of potato plants grown in pots and it was shown that under suitable conditions, one of which is a very high degree of soil moisture during part of the growing period, a high percentage of infected plants could be obtained regularly in small pots (7-inch diameter) (3). This partial standardisation of the method made it possible to compare the results of infection experiments carried out in pots with other means for testing viability of the Wart Disease organism.

A simple technique for testing *in vitro* the viability of the winter sporangium of *Synchytrium endobioticum* has been sought. The standard method of comparing the germination of samples of spores after subjection to various treatments has not been feasible because of the great difficulty experienced in inducing winter sporangia to germinate under laboratory conditions. Curtis (1) and others have with difficulty obtained a small percentage germination of winter sporangia kept in water for about ten weeks, and have actually observed the escape of zoospores, but such methods of germination are too uncertain and uncontrollable to provide a convenient technique for testing viability.

In the absence of a method for observing the actual germination of winter sporangia an estimate of the numbers of sporangia germinating in a given time might be made by counting the numbers of empty sporangia left. This has been attempted but the method is open to several objections. In the first place increase in the proportion of empty sporangia is slow. Counts made of the numbers of empty sporangia in three sets of one hundred removed at intervals from a tube containing sporangia in tap water at 18° C. are given in Table I.

The percentage of empty sporangia in a sample is small even after 13 weeks and the variation in different counts made at the same time is considerable. Direct evidence is needed that the presence of empty

Table I.

Percentage of Empty Sporangia developing in tap water at 18° C.

Date of Experiment: 5. vii. 21–6. x. 21.

	Original sample	After 3 weeks	After 6 weeks	After 13 weeks
Number of empty sporangia per hundred counted	4	14	9	14
	5	12	10	12
	0	9	12	21
Average percentage of empty sporangia	3	11·6	10·3	15·6

sporangia is due to germination and not to degeneration of the cell contents or to the activity of parasites which have recently been described by Köhler(4). Again fracture by mechanical means is very easily effected and produces empty sporangia resembling those which have germinated. Although it might be possible to develop a method of testing viability by observing the increase in numbers of empty sporangia it appeared likely to be tedious and unsatisfactory. A more rapid if less direct method was therefore sought.

The present communication describes experiments in which the reaction to certain stains is compared and correlated with the infecting power of sporangia which have been subjected to various treatments. Assuming that infecting power is a direct test of viability it is shown that there is a clear relationship between certain staining reactions of the sporangia and their viability, so that a rapid means of testing the latter becomes available.

Present Investigation.

Staining Reactions of Sporangia.

To find a method for testing the viability of winter sporangia of *Synchytrium endobioticum* it was necessary to compare the reactions of dead with those of living sporangia. Large numbers of winter sporangia fairly free from other organic matter were obtained by pressing decaying warts through a finely woven fabric mesh ($210\mu \times 210\mu$) and then centrifuging about twenty times. Of these sporangia, some were not treated, others were boiled for a few minutes in water and another sample was treated with commercial formaldehyde for two hours and then washed free from the reagent. The assumption was made that the untreated sporangia were alive and that the sporangia subjected to each of the drastic treatments described were dead.

The reactions of treated and untreated sporangia to a number of

stains, such as safranin, eosin, methylene blue, orange G, methyl violet, acid fuchsin etc. all in aqueous solutions, were observed. The thick brown sporangial wall not only hinders penetration but also makes it very difficult to observe the effect of the stain on the cell contents. By gently pressing down the cover-slip the sporangial walls were broken and the contents expressed into the stain while under observation.

The most decisive difference in the response of treated and untreated sporangia to the stains tested was found in the case of acid fuchsin 1–2 per cent. aq. The contents of untreated sporangia when pressed out into the stain slowly assumed a pale pink colour which gradually deepened. The expressed protoplasm generally showed considerable Brownian movement and its appearance suggested liquidity and mobility. In the case of treated sporangia the cell contents rapidly stained deep crimson and appeared coagulated, Brownian movement being absent. This difference in reaction was very striking and was observed in treated and untreated sporangia obtained from warts of very different ages. Further a number of samples of sporangia treated with different killing and fixing reagents for 24 hours and then washed, all stained rapidly and deeply when pressed out in acid fuchsin.

It therefore appeared possible that the acid fuchsin reaction of sporangia might be an indication of their viability. Extensive tests were carried out to discover what correlation could be found between the reaction of sporangia to acid fuchsin and their infecting power. Winter sporangia were subjected to a number of treatments varying greatly in intensity and type. These included subjection to various temperatures, and treatment with diverse chemicals such as a strong and a weak acid, a strong and a weak base, and various sterilising agents such as formaldehyde and phenol. Treated sporangia were examined by the acid fuchsin test and others similarly treated were used to inoculate potato plants grown in pots. The results were then compared.

Technique.

A measured quantity, 60 c.c., of dry warted material which had been pressed through a 3 mm. sieve was used for each treatment in the earlier experiments, *i.e.* the temperature series and sulphuric acid Set I. For sulphuric acid Set II and for subsequent experiments a much smaller quantity of sporangia was prepared by pressing wet warts through muslin, allowing to settle and pouring off the water. About 5–10 c.c. of the damp sporangial matter was used for each treatment. This was more easily wetted and convenient to use than the larger quantity though

it is liable to give slightly less infection. For each treatment the sporangial material was wetted as thoroughly as possible with 300 c.c. or when small quantities of sporangia were used with 200 c.c., using water in the temperature set and an aqueous solution of the particular chemical in the chemically treated series. After the chemical treatments the sporangia were freed from the reagent by repeated aqueous extractions followed by washing on fine silk spread over a filter funnel.

A. *Staining Test with Acid Fuchsin.* Treated sporangia separated from other organic material by pressing through fine voile and centrifuging were examined in acid fuchsin 2 per cent. aq. The coverglass was gently pressed so that the sporangial wall was broken and the contents expressed into the stain. At least 20 sporangia per test were examined in this way and their reaction noted. Sporangia which behaved in the same way as the controls were described as reacting with a "faint" stain. The intermediate group containing some faintly and some deeply staining sporangia together with some intermediate in reaction were classed as "intermediate," the relative proportions of each type varying considerably after different treatments. The third group consisted of sporangia which all stained deeply and rapidly.

B. *Infection Experiments in Pots.* When the larger quantity, 60 c.c., of sporangial material was used about five-sixths of it was mixed with each 15 lbs. of soil and the rest put directly on the tubers as they were planted. When the smaller quantity of sporangia was used it was all put on and around the tubers.

The soil consisted of a mixture of heavy soil from Rothamsted fields, sand and an organic material consisting of rotted cavings in the following proportions: soil 37·5 per cent., sand 50 per cent., cavings, 12·5 per cent. Each 15 lbs. of soil was divided and put into 5-inch earthenware pots containing sand to a height of about 1 inch, each standing in a saucer. A single tuber of the very susceptible variety Arran Chief was planted in each pot. The soil was kept fairly dry for two or three weeks until the shoots appeared above the surface, after which the soil was kept extremely wet with water standing almost continually in the saucers. Watering was effected by pouring water into the saucers and not directly on to the soil.

The potato plants were examined for Wart Disease after three months. In some cases the permanency of the effect of treatment on sporangia was tested by growing a second crop in the same soil after the completion of the first infection experiment. The soil from each set of five pots containing similarly treated sporangia was put in one large pot in

which three tubers were planted, watering being carried on as before. After three months the presence or absence of infection was noted.

Effect of Various Treatments on Staining Reaction and Infection Power of Winter Sporangia.

A. *Temperature.* The sporangial material in water was put on a water bath and raised to the required temperature at which it was maintained for one hour. The time taken to reach these temperatures varied from about 10 to 30 mins. Two sets of experiments were run, the second set repeating treatments at certain temperatures but using double the quantity of sporangial material so that twice the number of plants could be infected. In some cases a second crop was grown in the same soil after the completion of the first experiment.

Table II.

Effect of Temperature on the Staining Reaction and Infecting Power of Sporangia treated in Water.

Time: 1 hour.

Temp. ° C.	Stain in acid fuchsin	Infection in pot experiments: 1st crop: Number of plants	Number of plants infected	Percentage of plants infected	Infection in 2nd crop grown in same soil
Room temp.	Faint	9*	8	97	Present
		20	20		
30	,,	4	4	100	,,
40	,,	4	4	100	,,
45	,,	4	3	86	,,
		10	9		
50	Intermediate, majority faint	4	2	79	,,
		10	9		
55	Intermediate	2	0	8	,,
		10	1		
60	Intermediate, majority deep	4	0	5	,,
		15	1		
70	Deep	2	0	0	Absent
		5	0	0	,,
80	,,	5	0	0	,,
		5	0	0	,,

* In the first set some plants failed to grow because they were kept too wet before the sprouts had appeared above the surface of the soil. In later experiments this was remedied and very few failures were obtained.

Table II shows that when the sporangia stained faintly after treatment there was no apparent reduction in infection, when their reaction was intermediate a reduction in infection was apparent, while after

treatments which produced deeply staining sporangia no infection occurred. The permanency of the treatment was indicated by similar infection in the second crop.

B. *Sulphuric and Acetic Acids, Formaldehyde, Phenol, Ethyl Alcohol and Chloroform.* Winter sporangia were treated at room temperature with various concentrations of sulphuric acid for periods varying from 24 hours to 17 days. Two sets of infection experiments differing in the quantity of infecting material used, were put up side by side. In Set I infection was carried out as usual by using 60 c.c. of sporangial material, part of which was mixed with the soil and part applied directly on to the tuber while in Set II about 5 c.c. of sporangial material was used and this was all put directly on to the shoots of the tubers.

Tests were later carried out with acetic acid, formaldehyde, phenol, ethyl alcohol and chloroform, using the smaller quantity of sporangial material as in Set II of the sulphuric acid series.

The results are collected together in Table III which shows that in the sulphuric acid series infection was just as great whether the smaller or larger quantity of sporangial material was applied. The smaller amount was therefore used in subsequent experiments.

The relation between the acid fuchsin reaction of sporangia and their apparent infecting power was the same after each treatment as it was after subjection to various temperatures. Treatments after which sporangia stained faintly, as did the controls, did not appear to alter the infecting power of the sporangia, while intermediate treatments tended to reduce it and stronger treatments which affected sporangia so that their contents stained deeply and rapidly in acid fuchsin destroyed their infecting power altogether. As the latter may be regarded as a measure of sporangial viability a clear relationship is evident between the reaction to acid fuchsin and the viability of sporangia.

The results obtained in the second crop grown in one soil were similar to the first which favours the view that the effect on the sporangia was permanent.

Acetic acid proved less toxic than sulphuric acid which is worthy of particular note in view of its greater penetrating power and of the opposite results obtained by Loeb (6) experimenting with sea-urchin eggs.

C. *Alkaline Reagents.* Acid fuchsin is a disulphonic acid whose neutral salts are colourless while the acid itself and its mono-acid salts are red. It seemed probable therefore that if the contents of sporangia killed by the action of an alkaline reagent were expressed into acid fuchsin the neutral colourless salt would be formed and the sporangial

Table III.

The Effect of Various Chemical Reagents on the Reaction of Sporangia to acid fuchsin and on their infecting power.

Room Temperature.

Treatment	Concentration	Duration of treatment	Stain in 2 % acid fuchsin aq.	Infection in pot experiments: Set I, 1st crop: No. of plants	Set I, 1st crop: No. of plants infected	Set I: 2nd crop	Set II, 1st crop: No. of plants	Set II, 1st crop: No. of plants infected	Set II: 2nd crop	Percentage infection in 1st crop
Control	—	—	Faint	15	15	Present	10	10	Present	100
Sulphuric acid	*N*/500	24 hours	,,	5	4	—	5	5	,,	90
	N/500	7 days	,,	5	4	Present	—	—	—	80
	N/100	24 hours	,,	5	5	,,	5	5	Present	100
	N/100	7 days	,,	5	5	—	—	—	—	100
	N/10	24 hours	,,	5	4	Present	5	5	Present	90
	N/10	7 days	,,	5	5	,,	—	—	—	100
	N/5	24 hours	Intermediate	5	5	,,	5	5	Present	100
	N/5	3 days	,,	5	3	,,	—	—	—	60
	N	24 hours	Deep	5	0	Absent	5	0	Absent	0
	5 *N*	24 ,,	,,	5	0	,,	5	0	,,	0
Control	—	—	Faint	—	—	—	10	8	—	80
Acetic acid	*N*/500	24 hours	,,	—	—	—	5	5	—	100
	N/250	24 ,,	,,	—	—	—	5	4	—	80
	N/100	24 ,,	,,	—	—	—	5	5	—	100
	N/50	24 ,,	,,	—	—	—	5	5	—	100
	N/10	24 ,,	,,	—	—	—	5	5	—	100
	N/5	24 ,,	Intermediate	—	—	—	5	1	—	20
	N	24 ,,	,,	—	—	—	5	1	—	20
	5 *N*	24 ,,	Deep	—	—	—	5	0	Absent	0
Control	—	—	Faint	—	—	—	10	9	—	90
Formaldehyde	0·5 %	24 hours	,,	—	—	—	5	5	—	100
	1·0 %	24 ,,	Intermediate	—	—	—	5	0	—	0
	5·0 %	24 ,,	Deep	—	—	—	5	0	—	0
	10·0 %	24 ,,	,,	—	—	—	5	0	—	0
Control	—	—	Faint	—	—	—	15	12	—	80
Phenol	*M*/100	24 hours	,,	—	—	—	5	4	—	80
	M/10	24 ,,	Intermediate	—	—	—	5	0	—	0
	M	24 ,,	Deep	—	—	—	5	0	—	0
Control	—	—	Faint	—	—	—	10	9	—	90
Ethyl alcohol	100 %	1 hour	,,	—	—	—	5	5	—	100
	—	24 hours	Intermediate	—	—	—	5	1	—	20
	—	96 ,,	Deep	—	—	—	5	0	—	0
Chloroform	100 %	1 hour	Faint	—	—	—	4	4	—	100

Table IV.

The Effect of Alkaline Reagents on the Staining Reaction and Infecting Power of Sporangia.

Time 24 hours. Room temperature.

Treatment	Concentration	Appearance of sporangial contents in water	Stain in 2 % acid fuchsin aq.	Stain in 2·5 % methylene blue aq.	No. of plants	No. of plants infected	Percentage of plants infected
Sodium hydroxide	Control	Granular with rapid movement	Faint	Faint	15	10	67
	N/100	,, ,,	,,	,,	5	3	60
	N/10	,, ,,	,,	,,	5	2	40
	N	Translucent coagulated	Absent	Deep	5	0	0
	5 *N*	,, ,,	,,	,,	5	0	0
Ammonium hydroxide	Control	Granular with rapid movement	Faint	Faint	15	10	67
	N/100	,, ,,	,,	,,	5	3	60
	N	,, ,,	Intermediate	Intermediate	5	1	10
	5 *N*	Majority apparently solid, some liquid	,,	,,	5	1	10
	Sp. gr. 0·88	Translucent coagulated	Absent	Deep	5	0	0

contents would therefore remain colourless. Deep staining of the sporangial contents killed by an alkaline reagent might however take place in a basic dye. This possibility was tested with sporangia treated with the strong base, sodium hydroxide, and the weak base, ammonium hydroxide. Staining tests were carried out both in acid fuchsin and in the basic dye methylene blue. The appearance of the sporangia in water was also noted.

The contents of untreated sporangia and those treated with less concentrated hydroxides stained faintly in acid fuchsin and again in methylene blue while in water their contents appeared granular and showed considerable Brownian movement. Such sporangia produced infection in potato plants grown in pots.

After treatment with the more concentrated hydroxides the sporangial contents remained colourless in acid fuchsin but stained rapidly and deeply in methylene blue. These sporangia differed from the unaffected sporangia in that their contents had become clear and translucent, which could be observed without breaking the sporangial wall. This effect was never observed after any of the treatments previously described. When expressed into water the sporangial contents appeared coagulated and showed no Brownian movement. Treatments which caused sporangia to behave in this way completely destroyed their infecting power. Treatments producing an intermediate reaction tended to reduce infection.

It therefore appears that an indication of the viability of sporangia treated with strongly alkaline reagents can be obtained both from the appearance of their contents in water and from their reaction to methylene blue.

CONCLUSIONS.

The viability of the winter sporangium of the fungus *Synchytrium endobioticum* can be determined by means of staining tests. Sporangia which have been treated thermally or by various concentrations of reagents which are not alkaline in reaction, can by means of the acid fuchsin test be divided into three groups. Sporangia which, like the controls, stain faintly and slowly in acid fuchsin: these give a high percentage infection in potato plants grown in pots and therefore are alive. Groups of sporangia which are intermediate in reaction, some staining faintly, some deeply and rapidly, and some being intermediate; these tend to cause reduced infection indicating, that while some of them are alive others are probably dead. Finally groups of sporangia which all

stain deeply and rapidly in acid fuchsin: these produce no infection, which is presumptive evidence of death.

Sporangia which have been treated with alkaline reagents do not respond to acid fuchsin but this may be replaced by the basic stain methylene blue in which living sporangia stain faintly and dead ones deeply and rapidly. Further the appearance of the sporangial contents after treatment with alkaline hydroxides is an indication of their viability; the contents of living sporangia appear normal while dead ones are clear and translucent.

II. A METHOD FOR EXTRACTING WINTER SPORANGIA FROM SOIL.

The value of any method for testing the viability of the winter sporangium of *Synchytrium endobioticum* is likely to be greatly increased if it can be applied to sporangia which have been treated actually in soil. A simple method for extracting sporangia from soil, without affecting their viability, was therefore sought. A mechanical analysis of a sample of soil heavily contaminated with winter sporangia was carried out by the water settling method, with the idea that if the sporangia were sufficiently similar to each other in size and specific gravity they would be concentrated in one fraction of the soil. Most of the sporangia were recovered in the silt and fine silt but the remainder were scattered to a considerable extent through the other portions. The concentration of sporangia in any one fraction did not appear great enough to provide a method for recovering a sufficient number of sporangia to carry out viability tests, although Schander and Richter (11) have used a somewhat similar method for recognising contamination of soil by Wart Disease. A more rapid, efficient and less laborious method seemed desirable.

The probable difference in specific gravity of soil and winter sporangia appeared likely to provide the basis for a method of separation. An estimate was therefore made of the average specific gravity of the winter sporangium.

This was calculated by Stokes' formula as used in the mechanical analysis of soil (2) after estimating the rate of fall through water and the diameter of the average sporangium. To determine the rate of fall of sporangia in water a tube about 6 inches long, filled with water, was placed in a vertical position. Sporangia, first carefully separated from each other and from plant tissues by sieving through voile and centrifuging, were introduced in drops of water carefully placed on the surface of the water in the tube by means of a Pasteur pipette with as little disturbance as possible. The sporangia, after they had fallen a distance of about

4 cm. and apparently had assumed a steady rate of fall, came into the field of vision of a low power travelling-microscope placed horizonally and adjusted to observe the fall of the sporangium through 1 cm. The times in seconds taken by 18 sporangia chosen at random to fall the distance of 1 cm. in water were as follows: 37, 38, 6, 15, 20, 39, 57, 30, 30, 35, 22, 45, 74, 18, 23, 25, 51 and 69 giving an average of 35 secs. with a probable error of ± 4·3. The diameters in micro-millimetres of 12 sporangia also chosen at random were 55·2, 68·6, 46·0, 50·0, 43·0, 64·4, 40·0, 63·0, 50·0, 55·4, 44·2 and 56·6 giving an average of 56 μ with a probable error of approximately ± 2 μ. The average specific gravity calculated from these figures is 1·175 with a probable error of ± 0·021.

The specific gravity of ordinary arable soil as given by Warington (13) is 2·5 while Mosier and Gustafson (8) found it varied from 2·57 to 2·65 in estimations made on several different kinds of soil.

The difference in specific gravity of soil and sporangia appeared sufficiently great to allow of their separation by means of some liquid of intermediate specific gravity which should also have a low viscosity and should not affect the viability of the sporangium. Tetrachlorethane (sp. gr. 1·6) and chloroform (sp. gr. 1·5 approx.) both have a low viscosity and were tested for this purpose. The former, although not unpromising, gives off vapours which are very toxic to human beings, so that chloroform was preferred. Air-dry soil passed through a 3 mm. sieve was heavily contaminated with sporangia, and passed through voile with a mesh of 210 × 210 μ. The sporangia passed through while larger pieces of organic matter were left behind. About 20 gm. of this soil were then shaken up with chloroform and allowed to stand until the soil had settled at the bottom. A separation was immediately made into three portions which consisted of:

(*a*) Particles floating on top of the chloroform. These were mostly sporangia together with small pieces of organic matter.

(*b*) Particles actually in the chloroform which were filtered off. Large numbers of sporangia together with traces of organic matter were found in this portion.

(*c*) The residue consisting chiefly of soil with which very few sporangia had settled.

A second extraction of the residue gave a few sporangia in the chloroform but far the greater quantity were obtained in the first extraction.

The method consists therefore of careful sieving of contaminated soil, to reduce the size and quantity of organic matter which will be separated

with the sporangia, followed by extraction with chloroform. The whole of the chloroform is pipetted off as soon as the soil has settled and is then filtered. The filtrate is examined for sporangia.

Sporangia separated in this way and examined by the acid fuchsin staining test showed no effect of the chloroform on the viability of the sporangium. Table III shows that sporangia treated with chloroform for one hour still stained faintly and slowly as did untreated sporangia, and that their infecting power appeared unaffected in pot experiments.

This method although it has been used successfully in extracting sporangia from soil as it occurs in the field, *i.e.* in the workhouse grounds at Ormskirk, is more applicable in the case of small quantities of heavily infected soil in the laboratory. It has been tested in conjunction with pot experiments in the following manner. Contaminated soil treated with various chemicals has been put in pots. Small quantites of soil from each pot have been still more heavily contaminated with sporangia, wrapped up in bags made of voile and sunk in the pots for a month, after which the bags were removed and tubers planted in the pots. Sporangia extracted by chloroform from the soil in the bags were examined by the acid fuchsin staining tests and showed little or no deep staining, indicating that they were still capable of causing infection. The results of the pot experiments showed this to be the case.

III. THE EFFECT OF TEMPERATURE ON THE VIABILITY OF THE WINTER SPORANGIUM.

Introduction.

Steam has been found effective in eradicating *Synchytrium endobioticum* from soil by several workers, notably Gimingham and Spinks (2), Kunkel and Orton (5) and by investigators at Rothamsted (9). Except for this fact practically nothing is known of the effect of temperature on the viability of winter sporangia. Information of this type is needed as a basis for pot and field work and is interesting in view of the high resistance of the sporangium to other toxic agents.

Temperature and Viability.

Technique.

Sporangia were obtained fairly free from other organic matter by pressing decaying warts through a finely woven fabric and centrifuging several times. A small quantity of damp sporangial material was introduced into distilled water already heated to the required temperature

in an electric oven. The sporangia were maintained at this temperature for definite lengths of time, then filtered off rapidly and their viability determined by the acid fuchsin test. Counts of three sets of twenty sporangia were made after each treatment and the numbers reacting to acid fuchsin by "faint," "intermediate" and "deep" staining recorded.

As these groups merge into each other it is sometimes a little difficult to ensure prefect discrimination between them and so obtain exact percentages, but the staining reaction affords a means for a general comparison of the viability of sporangia after different treatments.

Dry sporangia subjected to different temperatures were treated in the same way as wet sporangia, except that they were first air dried and then kept dry during treatment.

Temperature and Viability of the Wet Sporangium.

The reactions of winter sporangia after subjection in the wet state to different temperatures for varying lengths of time are shown in Table V, which gives the actual and average numbers of sporangia which produce a faint, intermediate and deep stain with acid fuchsin.

Diagram I, in which time is plotted on the logarithmic scale, represents the average result of each treatment. The central point of each square marks the time and temperature of each reading, and the intensity of shading indicates the proportion of sporangia which gave a faint, intermediate and deep stain with acid fuchsin. A comparison of the relative viability of sporangia after different treatments is thus readily made. The sporangia all stain deeply, that is, they are probably all dead after treatment at 90° C. for 5 minutes, 80° C. for 15 minutes, 70° C. for 1 hour and 60° C. for 8 hours.

Temperature and Viability of the Dry Sporangium.

Observations were made on the effect of high temperature on the staining reaction of dry sporangia to acid fuchsin.

The results showed, Table V, that a very much higher temperature was required to kill sporangia in a dry condition than when wet. Thus while 15 minutes at 80° C. or 1 hour at 70° C. is enough to kill sporangia in the wet state, $1\frac{1}{2}$ hours at 80° C. and 20 hours at 70° C. is not sufficient to kill them when dry. This fact, which is common to most micro-organisms, is of importance in any practical application which may be made of these results. In heating soil for the purpose of killing the sporangia of *Synchytrium endobioticum* it is very important that the soil should be uniformly damp.

Table V.

The Effect of Temperature on the Reaction to Acid Fuchsin of Wet Sporangia.

Temp. ° C.	Time	Staining reaction of Sporangia			Average		
		Faint	Medium	Deep	Faint	Medium	Deep
40	1 hour	20	0	0			
		20	0	0	20	0	0
		19	1	0			
	8 hours	18	0	2			
		17	2	1	18	1	1
		19	1	0			
	16 „	20	0	0			
		20	0	0	19	1	0
		17	3	0			
	24 „	14	4	2			
		13	5	2	15	4	1
		17	3	0			
50	15 mins.	19	0	1			
		18	2	0	19	1	0
		19	1	0			
	30 „	20	0	0			
		20	0	0	19	1	0
		16	4	0			
	1 hour	18	2	0			
		19	1	0	17	3	0
		15	5	0			
	2 hours	18	1	1			
		16	1	3	16	2	2
		15	4	1			
	8 „	15	5	0			
		7	11	2	10	8	2
		9	8	3			
	16 „	3	12	5			
		8	5	7	5	10	5
		4	13	3			
	24 „	4	13	3			
		2	4	14	3	10	7
		1	14	5			
60	5 mins.	16	2	2			
		18	2	0	17	2	1
		19	1	0			
	15 „	8	9	3			
		6	14	0	9	10	1
		12	7	1			
	30 „	10	8	2			
		6	11	3	8	10	2
		7	12	1			
	1 hour	7	10	3			
		1	6	13	4	8	8
		3	7	10			
	2 hours	2	9	9			
		0	4	16	1	7	12
		2	7	11			
	8 „	0	0	20			
		0	0	20	0	0	20
		0	0	20			

Table V (*continued*).

Temp. ° C.	Time	Staining reaction of Sporangia: Faint	Medium	Deep	Average: Faint	Medium	Deep
60	16 hours	0	0	20			
		0	0	20	0	0	20
		0	0	20			
	24 ,,	0	0	20			
		0	0	20	0	0	20
		0	0	20			
70	5 mins.	2	7	11			
		1	3	16	1	5	14
		1	6	13			
	15 ,,	2	5	13			
		0	1	19	1	4	15
		0	5	15			
	30 ,,	0	5	15			
		0	4	16	0	5	15
		0	6	14			
	1 hour	0	0	20			
		0	1	19	0	0	20
		0	0	20			
	2 hours	0	0	20			
		0	1	19	0	0	20
		0	0	20			
80	5 mins.	0	1	19			
		0	1	19	0	1	19
		0	1	19			
	15 ,,	0	1	19			
		0	0	20	0	0	20
		0	0	20			
	30 ,,	0	0	20			
		0	0	20	0	0	20
		0	0	20			
	1 hour	0	0	20			
		0	0	20	0	0	20
		0	0	20			
90	5 mins.	0	0	20			
		0	0	20	0	0	20
		0	0	20			
	15 ,,	0	0	20			
		0	0	20	0	0	20
		0	0	20			
	30 ,,	0	0	20			
		0	0	20	0	0	20
		0	0	20			
	1 hour	0	0	20			
		0	0	20	0	0	20
		0	0	20			
100	5 mins.	0	0	20			
		0	0	20	0	0	20
		0	0	20			
	15 ,,	0	0	20			
		0	0	20	0	0	20
		0	0	20			

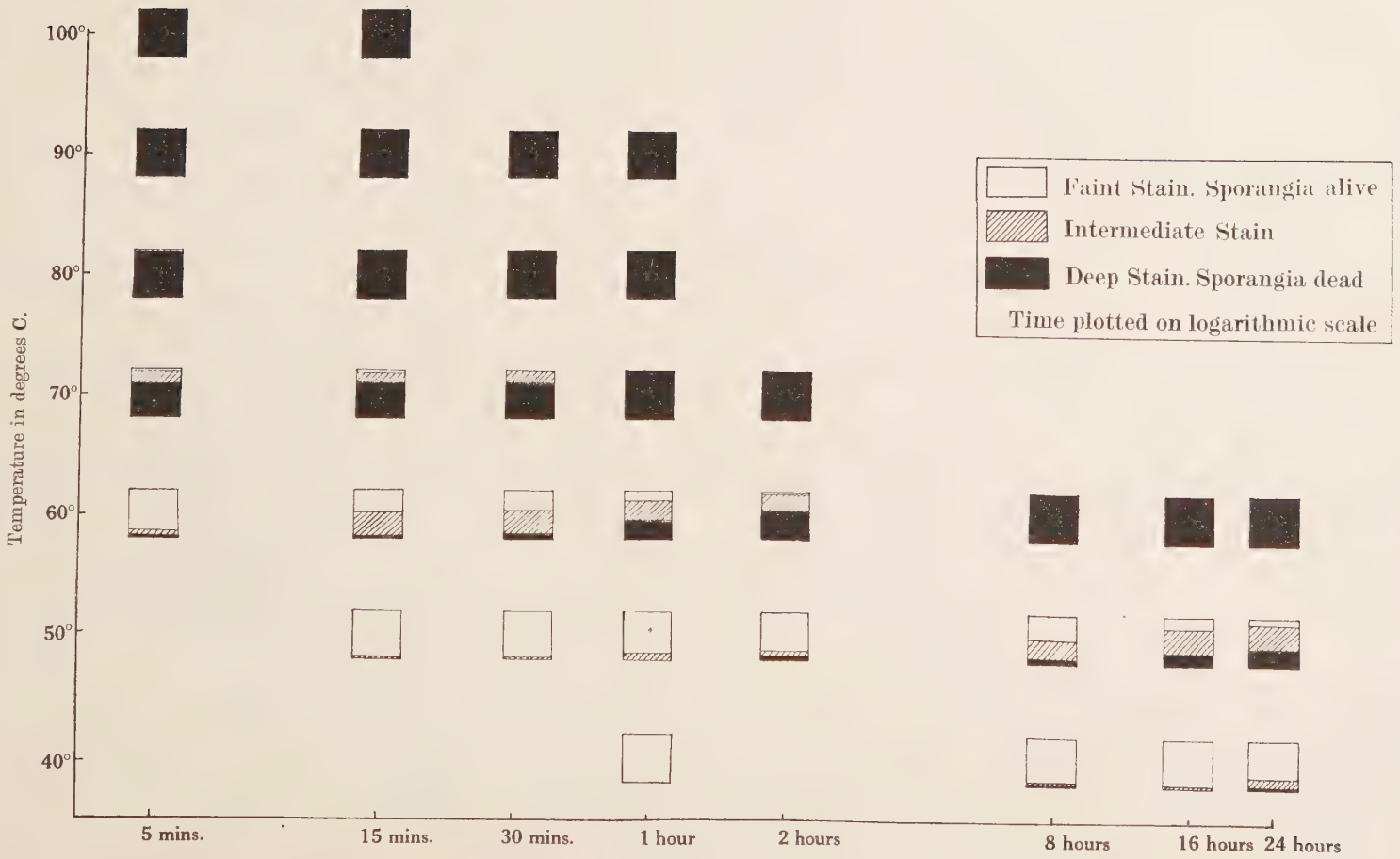

Diagram I. The viability of the winter sporangium.

Table VI.

The Effect of Temperature on the Reaction to Acid Fuchsin of Dry Sporangia.

Temp. °C.	Time	Staining reaction of Sporangia			Average		
		Faint	Medium	Deep	Faint	Medium	Deep
75	½ hour	13	6	1			
		13	5	2	14	5	1
		15	5	0			
75	20 hours	7	9	4			
		12	6	2	8	8	4
		6	8	6			
80	1½ „	19	1	0			
		19	1	0	19	1	0
		20	0	0			
90	5 mins.	18	2	0			
		16	3	1	17	2	1
		18	1	1			
90	15 „	16	4	0			
		16	4	0	17	3	0
		20	0	0			

SUMMARY.

A staining method for testing the viability of the winter sporangia of *Synchytrium endobioticum* is described. The sporangial contents are pressed out into acid fuchsin or after treatment by a strongly alkaline reagent into methylene blue. The staining reactions have been correlated with the results of infection experiments in pots. Sporangia which, like the controls stain faintly, produce a high percentage infection and are therefore alive. Those which stain deeply and rapidly produce no infection and are presumably dead. There is an intermediate group in which some sporangia stain deeply and some are intermediate in reaction. This group tends to give less infection than the controls.

A method whereby sporangia which have been treated in soil may be extracted without affecting their viability is described. The method depends on the difference in specific gravity of sporangia which has been determined as about 1·17 and of soil which is in the region of 2·5. The sporangia are extracted by means of chloroform (sp. gr. 1·5 approx.), which does not affect their viability.

A study of the relation of temperature, time and viability shows that treatment for 5 minutes at 90° C., 15 minutes at 80° C., 1 hour at 70° C. and 8 hours at 60° C. have a similar effect in killing all the sporangia.

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MOISTURE MOVEMENT IN WOOD

I. THE TRANSFER OF MOISTURE BETWEEN TWO DISCS OF WOOD

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(With 5 Text-figures.)

Introduction.

The following paper describes some experiments which were carried out in connection with the artificial seasoning of timber and may serve as an introduction to later papers dealing with the factors that appear to influence the passage of moisture through wood.

Owing to differences in the structure of timber and to variations in its relation to "external" conditions the investigation of conduction phenomena in this substance is complicated by the introduction of variables, which however fall conveniently into two groups; those which depend upon external conditions such as temperature, relative humidity and barometric pressure and those which depend upon the material itself, in which may be included such factors as dimension, density, structure and direction of grain.

A thorough knowledge of the influence of these variables on the rate of drying and on passage of moisture through wood is required in order to place the practice of timber seasoning on a sound basis.

Although wood is by no means a homogeneous or isotropic substance, the essential features of moisture movement through it do not differ fundamentally from those of other conduction phenomena, such for example as the diffusion of gases or of salts in water or the conduction of heat. There are several possible methods by which such problems can be investigated, which in the case of conduction of moisture through wood can be grouped as follows:

I. Those in which a steady flow of moisture is established by maintaining a constant difference of conditions at two parallel faces.

II. Those dealing with the wetting (or drying) of a piece of wood

of initial uniform moisture distribution, where the conditions at the surface are kept constant.

III. Those dealing with the exchange of moisture between two contiguous blocks which initially were of a different moisture content of uniform distribution.

In experiments based on the above methods the principal measurements would comprise determinations of the rate of flow of moisture, the rate of loss or gain of moisture, the moisture gradient and its variation with time.

It will be seen from the following brief description of the present experiments that they fall under the last of the three groups mentioned above. Two similar discs of wood of different but known moisture content were selected and were kept in close contact for a given period, after which the change in the moisture content of each was determined. A whole series of such determinations, using the same or different pairs of discs, was made for various time intervals and for two different thicknesses of material.

This particular line of attack on problems connected with conduction has been pursued by investigators in other fields.

Graham(4), in 1863, employed this method in an investigation on the diffusion of gases. He filled the upper nine-tenths of a vertical cylinder with the lighter and the lower tenth with the heavier of a pair of gases. After a suitable interval he analysed the gas in the upper tenth of the cylinder and thereby obtained the relative rates of diffusion of different gases. Loschmidt(6), in 1870, performed similar experiments with greater precision, but with this difference that each gas filled one-half of the cylinder.

Voit(7), in 1867, using a similar method, investigated the diffusion of salts in water. He filled the lower half of a glass vessel with a solution of a salt and the upper half with water. By optical means (refraction) he determined the time taken for the salt solution to reach a given concentration at different levels in the vessel. J. C. Graham(3), in 1905, also studied the diffusion of salts but the method he adopted differed from that of Voit in that he determined directly, by means of density measurements, the concentration of the solution at a given moment at different levels. Weber's(8) technique in dealing with the same problem was to float a dilute salt solution on the top of one of greater concentration. At suitable intervals he determined electrically the concentration of the salt at the top and at the bottom of the column of liquid.

Although Buckingham(1) carried out a few experiments on these

lines during his investigations on moisture movement in soils, he made no attempt to obtain a value for the coefficient of conductivity of water in soil.

Data derived from experiments of the above type are generally put into such a form as to permit of a ready comparison with the law of diffusion first enunciated by Fourier (2) in connection with the conduction of heat. As adapted to moisture movement in wood this law would read thus: the rate of moisture movement in wood should be directly proportional to the moisture gradient, and the direction of movement should be from regions of high to regions of low concentration and at right angles to planes of uniform moisture content. Expressed mathematically this law takes the form of the differential equation

$$\frac{du}{dt} = k\frac{d^2u}{dx^2}$$

where t denotes time, u concentration, x distance, and k the coefficient of conductivity.

In order to apply the law of diffusion to experimental data the general equation given above has to be solved so as to satisfy the particular conditions of the experiment. In the present instance, for example, the conditions are that the two discs of wood are of the same dimensions, that the moisture in each is initially of uniform distribution, and that they lose or gain moisture only through the faces which are in contact. A solution of the general equation which satisfies these conditions gives the following:

$$\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2} = \frac{8}{\pi^2}\left\{e^{-\left(\frac{\pi}{l}\right)^2 kt} + \frac{1}{3^2}e^{-\left(\frac{3\pi}{l}\right)^2 kt} + \ldots\right\},$$

where D is the difference, S the sum of the moisture contents (gm.) of the two discs at the time t hours, l (cm.) the combined thickness of the two discs, u_1 the initial moisture concentration (gm. per cm.^{3}) of the wetter and u_2 that of the drier of the discs, k the coefficient of moisture conductivity, and e the Napierian base.

At the time $t = 0$ the value of either side of the equation will be unity and at time infinity it will be zero.

Material.

The material used for the experiments was rotary cut Birch veneer of two thicknesses, 1/8th inch and 1/16th inch. This species was chosen on account of its comparatively uniform structure; veneer material on

account of the uniformity of thickness and the comparatively short time required for it to reach equilibrium in a damp or dry atmosphere.

Apparatus.

The apparatus used for the experiments consisted of a balance, a number of desiccators, an oven for maintaining a constant temperature and two clamps for holding the pairs of dry and damp discs in contact.

Each clamp possessed two heavy brass plates between which three or four pairs of discs could be inserted. Sufficient pressure to obtain even contact of the discs could be produced by means of winged nuts operating on the screwed rods which served to hold the apparatus

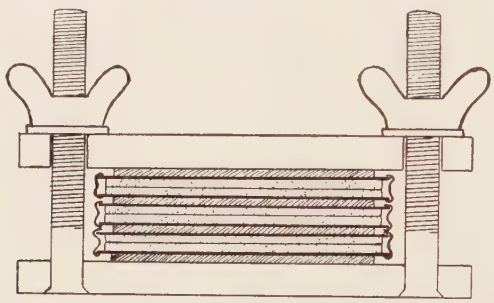

Fig. 1. Diagram of clamp. Pairs of discs stippled. Wooden spacers shaded.

together. In order to reduce moisture losses and to facilitate handling, each pair of discs was enclosed by a pair of thin brass plates and the edges protected from evaporation by means of a rubber band placed round the whole. To prevent the overlapping edges of the rubber bands affecting the pressure distribution on the discs, wooden separators of a slightly less diameter were inserted between each pair when the clamp was being loaded, as shown diagrammatically in the accompanying text-figure.

Procedure.

The veneer was cut into a number of discs about 4 inches in diameter. One half were placed in desiccators in which the atmosphere was kept dry by a solution of concentrated sulphuric acid, and the remainder in desiccators in which the air was kept moist, at about 95 per cent. relative humidity, by a dilute solution of sulphuric acid. When, after about a week, the discs had approximately attained moisture equilibrium with the atmosphere in the desiccators, they were removed one at a time and reduced to the size of a circle of slightly smaller diameter than that of the thin brass plates mentioned above. They

were then replaced in their respective desiccators for a further period of at least a week. At the end of this time it was found that any change in weight of the discs was within the experimental error of weighing which was about ± 0·002 gm.

As the first step in a series of experiments, the requisite number of discs were removed from the dry and damp desiccators, weighed as rapidly as possible and then replaced. At intervals of two minutes pairs of discs, one from a damp and the other from a dry desiccator, were placed between two of the brass plates and the whole bound with a rubber band which was made from a strip cut from a piece of sheet rubber.

One, two, or three pairs of discs as the case might be, protected from evaporation in this manner and separated by the wooden spacers, were then placed in a clamp which had been kept in an oven at 25° C., and screwed securely together. The loaded clamps were then replaced in the oven for the time decided upon for the particular experiment. At the end of this period the discs were removed from the clamp and weighed in the same order and at the same time interval in which they were assembled. They were then either replaced in the clamp for a further period of moisture transfer or else measured. The diameter was calculated from eight calliper readings and the thickness from the average of several readings of a micrometer screw. With the subsequent determination of the oven-dry weight, all the necessary experimental data had been obtained.

With most of the 1/8th inch material the experiments were repeated several times, and in each case the discs were brought back again to their respective initial conditions. In these circumstances the oven-dry weights were only obtained after the last repetition. The object of repeating the experiments was to learn to what extent experimental factors, apart from differences in the material, affected the results.

A summary of the experiments for the two sizes of material and for different time intervals is given in Table I.

The data derived from the experiments were tabulated so as to give all the necessary values for calculating $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$, which is the left-hand side of the equation given on p. 39. As these tables are somewhat extensive only one, Table II, is included in the text.

The mean value obtained for the term $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ together with the probable error for the different periods of time for the two thick-

nesses of material is given in Table III. These values are shown in the form of a graph in Figs. 2 and 3, the dotted line representing the smooth curve drawn through the experimental points. Values taken from these two curves plotted against log. time are again shown in Fig. 4.

This latter method of representation brings out more clearly the effect of thickness and other factors on the course of the moisture exchange.

Discussion of Results.

Before a discussion of the moisture transfer in the above experiments can be entertained, it is necessary to determine what its course would

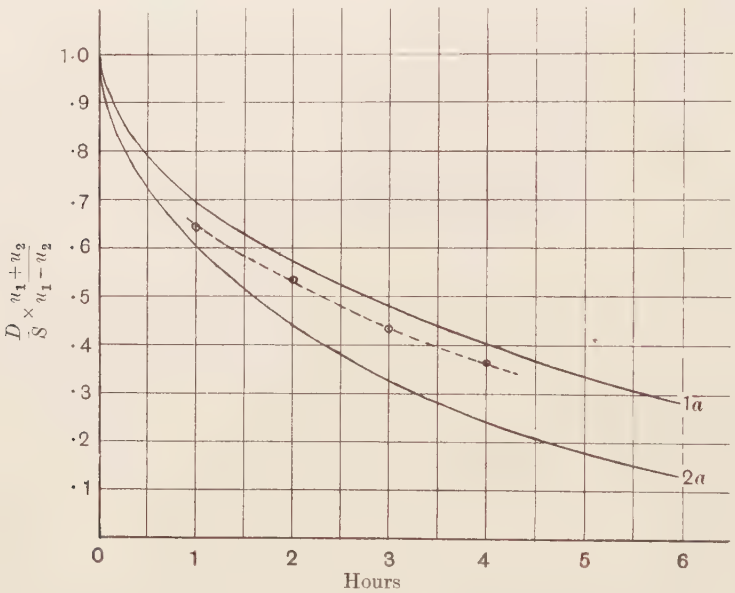

Fig. 2. Relation between $\frac{D}{S} \times \frac{u_1+u_2}{u_1-u_2}$ and time for 1/16 inch material. The full line curves 1 *a* and 2 *a* represent the theoretical relation for material of the same thickness in which the values of the coefficient of conductivity are respectively 0·00185 and 0·00320 gm. cm.^{3} hours^{-1}.

be under similar conditions if the law of diffusion applied. A comparison of the practical and theoretical values should then be of direct use in showing to what extent the law of diffusion could be usefully employed in the investigation of the numerous factors which may influence moisture movement in wood.

With this object in view the theoretical amount of moisture transfer,

which is represented in these experiments by the fraction $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$, was determined from the given equation at successive intervals of time for material of the two thicknesses used in the present investigation, employing two arbitrary values for the coefficient of conductivity, one greater and the other less than the average obtained from the experiments themselves.

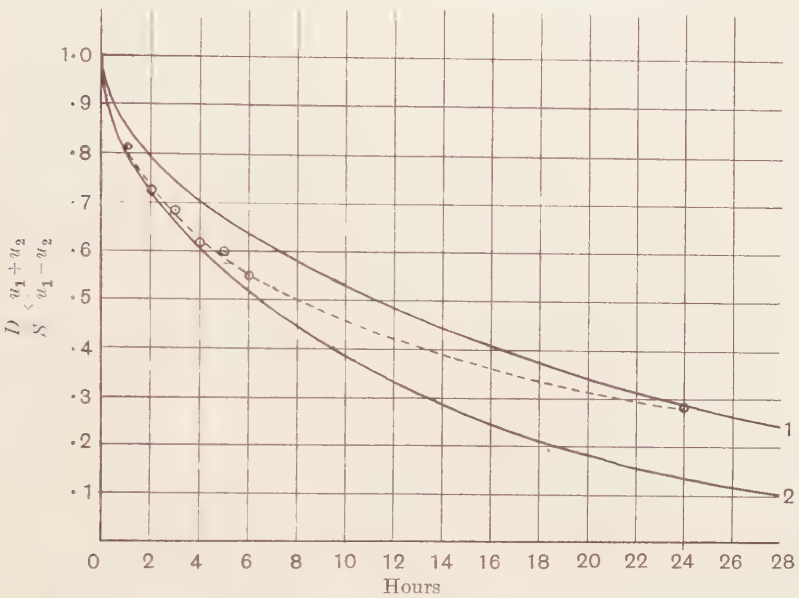

Fig. 3. Relation between $\frac{D}{S} \times \frac{u_1+u_2}{u_1-u_2}$ and time for 1/8 inch material. The full line curves 1 and 2 represent the theoretical relation for material of the same thickness in which the values of the coefficient of conductivity are respectively 0·00185 and 0·00320 gm. cm.^{3} hours^{-1}.

The results of these calculations are embodied in Table V. For comparison with the experimental values they are shown in graphical form by the full line curves in Figs. 2 to 4.

An examination of Figs. 2 and 3 shows that the rate of moisture exchange between the discs falls off more rapidly than is demanded by the theory of diffusion. This falling off would be accounted for if the moisture movement in wood was dependent upon the moisture content as well as upon the moisture gradient. In the present experiments it would appear that this decrease is due to the progressively diminishing moisture content of the damper disc since it has been definitely shown

by experiments to be described later that below the fibre saturation point a much greater potential is required to produce the same rate of flow in dry as in damp wood. Thus it will be seen that in order to obtain agreement with the law of diffusion an additional term will have to be introduced into the theoretical equation involving the change of moisture content with time.

A similar phenomenon was encountered by Lederer(5) in 1924 when analysing the drying rates of soaps. His experiments showed consistently that when a mean value was taken for the coefficient of conductivity over the whole drying period, the rate of moisture loss was somewhat greater than that demanded by the theory of diffusion in the earlier stages of drying but in the later stages somewhat less. The figures published indicate that moisture movement in soap is influenced to a much smaller degree by moisture content than is the case for wood.

It now remains to be considered how the course of moisture exchange would be affected theoretically by differences of thickness and by variations in the coefficient of conduction independent of those due to the changes in the moisture content discussed in the previous paragraphs.

For any given stage in the course of the moisture transfer there is naturally a definite value for $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$, which alters from unity at the commencement of the transfer to zero at the close. For every value of $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ there must be corresponding fixed values for the exponents $\left(\frac{n\pi}{l}\right)^2 kt$, of e, whatever the experimental conditions may be. It follows at once from the form of the exponents that the time (t) for the moisture transfer to arrive at a given stage must vary directly as the square of the thickness (l) of the pair of discs and inversely as the coefficient of their conductivity (k).

In the present instance the ratio of the squares of the two thicknesses of material used is as 4·05 to 1·00, which theoretically should be the same as the relative rates of their moisture exchange. From an examination of Fig. 4 it is seen that the dotted curves are separated by an amount 0·553, the logarithm of 3·57, which is a figure within 12 per cent. of the theoretical value of 4·05. After dealing with the actual calculation of the coefficient it will be shown that certain corrections will have to be applied to take account of the somewhat greater moisture conductivity of the thinner material.

It is assumed here that change in the coefficient with the moisture

content will not appreciably affect the relative rates of moisture transfer for the discs of the two thicknesses, since both should be affected to the same degree by this variation.

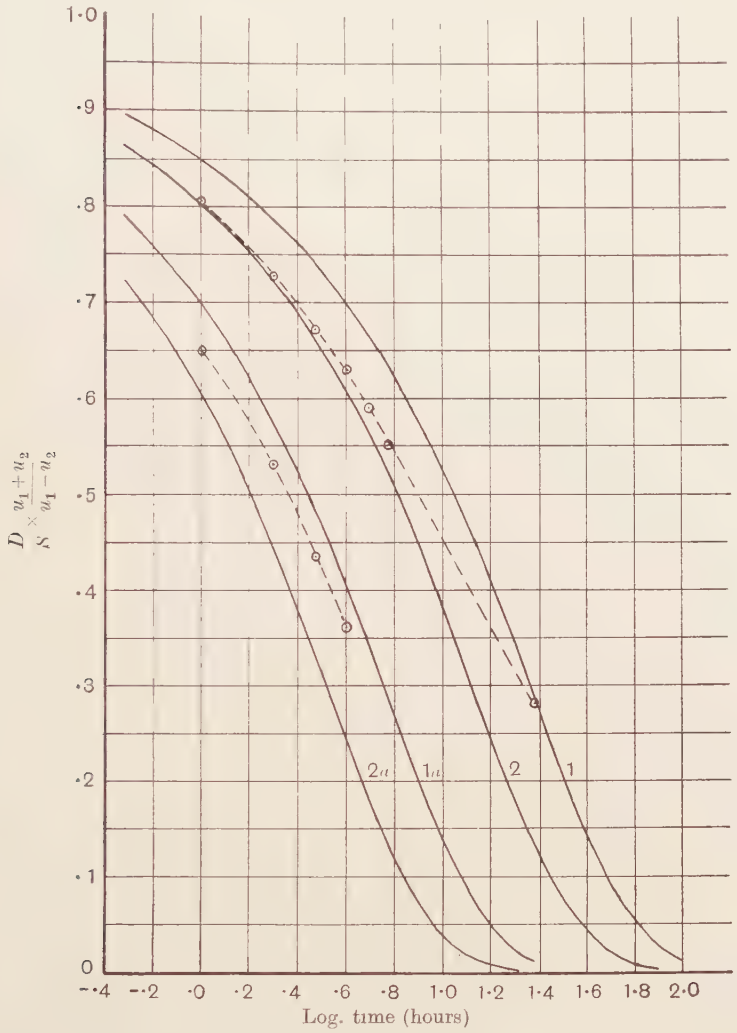

Fig. 4. The same curves as in Figs. 2 and 3 plotted against the logarithm of time, numbering as in Figs. 2 and 3.

The calculation of the coefficient of conductivity of wood from these or other experiments is beset with numerous difficulties even apart from those which may result from variations in the general structure, in its

density and in its chemical composition. First, there is the partial dependence of the rate of moisture movement upon the moisture content which has been alluded to previously, due to which any determination of the coefficient would only represent its mean value up to that moment but not its actual value at the time. Secondly, the volume of wood below the fibre saturation point—the condition applying to these experiments—changes with the moisture content. In the present experiments the initial concentration of the discs was based on dimension measurements made at the conclusion of a series of experiments. At the end of a 24-hour period, alteration in the volume of the discs due to changes in their moisture content would result in an error of the order of $1\frac{1}{2}$ to 2 per cent. in the coefficient of conductivity. Finally the value of the coefficient depends upon the units in which the moisture gradient is expressed, which in the case of wood can be measured in three different ways, each of which will affect its value to a different degree. In the present experiments the moisture was expressed on a concentration basis along the gradient, that is, as gm. per cm.^{3}, but it could have been given in terms of percentage of the dry weight, or finally, as there is a definite relation between moisture content and the partial vapour pressure, in terms of vapour pressure.

The first method has the advantage in that the coefficient is obtained directly in C.G.S. units. On the other hand, variation in the specific gravity of the samples will affect the value of the coefficient, depending as it does on the moisture gradient, since variation of pore-space to wood substance will affect the values for the moisture content of the wood along the gradient when the latter is expressed on a concentration basis. When the moisture content is expressed as a percentage of the oven-dry weight, the moisture gradient will not be affected by specific gravity, so in this case another factor would have to be introduced to account for any influence specific gravity might have on the rate of moisture movement in wood. The last method, involving the use of vapour pressure, is likely to be the most useful when investigating the effect of temperature on moisture movements.

On the purely experimental side there are numerous sources of error, among which may be mentioned differences in the dimensions of the two members of a pair of discs, variations in the perfection of contact between the discs and moisture losses during their manipulation. The last was compensated for by proportioning the loss between the two discs according to their moisture contents, as may be seen from examining the figures in Table II.

Certain figures however were obtained for the coefficient of conductivity which are not without value in spite of the difficulties enumerated above. Using values for $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ taken from the curves in Figs. 2 and 3 the coefficient k was calculated from the equation on p. 39 by

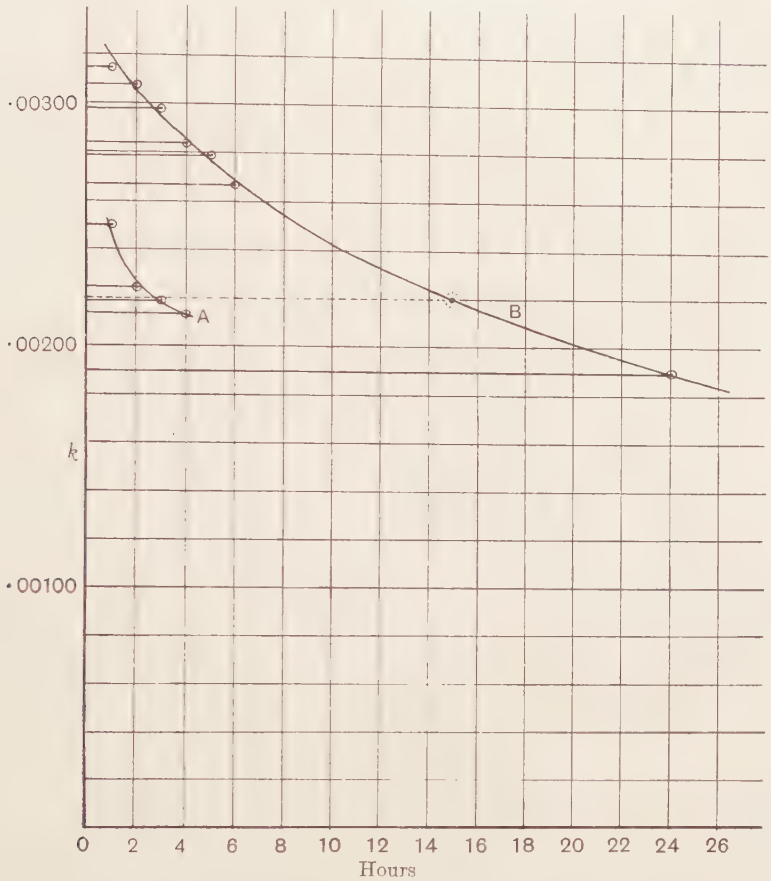

Fig. 5. Curves illustrating the change in the value of the coefficient of conductivity with time. Curve A, 1/16 inch material. Curve B, 1/8 inch material.

the method of successive approximations, for increasing lengths of time from the start of the moisture transfer.

The values obtained for the material of the two thicknesses are given in Table IV, the last column of which shows the variation to be expected in the coefficient due to the probable error in the determination of

$\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$. It is seen at once from these figures, as has already been inferred from the curves, that there is a marked falling off in the value of the coefficient with time, which is brought out very clearly by the curves in Fig. 5, which are drawn through the ends of the horizontal lines representing the mean value of the coefficient over the length of time to which the calculation applies.

In order to avoid complications due to these changes in the coefficient, due to changes in moisture content, when comparing the conductivities of material of different thicknesses, comparisons will have to be made at corresponding stages of moisture transfer, that is, at times when $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ has the same value. In the present instance it was found that over the period in which comparisons could be made the coefficient of the thinner material on the average was 1·07 times greater than that of the thicker. It follows from this that, to obtain a correct figure for the effect of thickness for these experiments, the periods taken for the thinner discs to reach given stages of the moisture transfer should be increased 1·07 times in order to reduce the coefficient of conductivity to the same value as it has for the thicker material. With this correction the rate of moisture transfer for the thicker material will be 3·43 and not 3·57 times greater than that for the thinner material, which make the difference in the times of transfer differ by 15 per cent. instead of 12 per cent. from theoretical requirements.

The experiments were not sufficiently extensive to determine how far this difference was due to defects in technique or to the presence of other factors of which no account had been taken.

An attempt was made to obtain an idea of the extent of the errors due to technique apart from variations in the material itself. In Table VI are set out the means for $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ for two groups of seven experiments which were repeated three times. The differences between these means and their average for the different periods of time are approximately twice as great as the probable error for the complete series, which seems to indicate that errors of technique are as great or even greater than the variations due to differences in the character of the material.

With regard to the effect of temperature on the rate of exchange of moisture only a few experiments were conducted; these would not have been mentioned but for the fact that they illustrate some remarks that

have been made on the advantage which would be gained by the method of expressing moisture gradients in terms of vapour pressure. The experiments were four in number, carried out with 1/8 inch material at a temperature of 35° C., for a period of one hour. Except for temperature, there were no differences in the conditions of these experiments from those carried out at 25° C. The mean value of $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ was found to be ·756, which gave a value of ·00496 for the coefficient of conductivity. Thus the rise of 10° C. in the temperature raised the coefficient to 1·57 times its value (·00315) at 25° C.

Since the initial moisture conditions were not appreciably different from those of the experiments carried out at 25° C. this increase cannot be due to any great alteration in the moisture concentration gradient but must have its seat in the vapour pressure gradient parallel to that of the moisture concentration, which latter will not be affected, or at least not to anything like the same extent, by changes of temperature. The actual ratio of the saturated vapour pressures at temperatures of 25° C. and 35° C. is as 1 to 1·77, from which the increase in the rate of moisture transfer, namely 1·57, differs by only 12 per cent. Other experiments, to be described in a later paper, show that the relation between moisture movement and vapour pressure is very much closer than indicated by the above figure.

In conclusion, it appears that although the law of diffusion is not strictly followed yet the differences between theory and experimental results are not sufficiently great to prevent its application in the analysis and interpretation of experiments dealing with the factors which may influence the moisture movement in wood.

Summary.

1. A number of experiments were carried out on the exchange of moisture between discs of 1/8th inch and 1/16th inch Birch veneer below the fibre saturation point.

2. It appears from these experiments that the rate of moisture movement in wood below the fibre saturation point is dependent, not only upon the moisture gradient, but also upon the moisture content. But for this limitation, fair agreement was obtained with the law of diffusion. The values obtained for the coefficient of conductivity have no definite significance as they represent mean values over a period.

3. The rate of moisture exchange between the discs was found to be approximately proportional to the square of their combined thickness

and was within 15 per cent. of the demands of the law of diffusion for these particular experiments.

4. From the few experiments carried out at a higher temperature it would appear probable that the change in the rate of moisture movement with rise in temperature is proportional to the change in the vapour pressure of water.

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Table I.

Summary of the number of experiments carried out. In the case of the 1/8*th inch material the numbers on the same horizontal line are experiments carried out with the same pairs of discs. Experiments repeated a first or second time are denoted by* r_1 *and* r_2 *respectively.*

Material	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	24 hours
1/16″	29	16	12	14	—	—	—
1/8″	10 r_1 10	—	—	—	—	—	—
1/8″	—	—	—	16	—	—	—
1/8″	10 r_1 11 r_2 7	— — —	12 r_1 12 r_2 7	— — —	11 r_1 12 r_2 7	— — —	— r_1 12 r_2 7
1/8″	— — —	12 r_1 12 r_2 8	— — —	12 r_1 12 r_2 7	— — —	11 r_1 12 r_2 7	— r_1 12 r_2 7

Table II.

Data derived from experiments summarised in Table I.

Exp.	Thickness (cm.)	Diameter (cm.)	Volume (c.c.)	Weight (gm.) Oven dry	Weight (gm.) Initial	Initial moisture	Initial moisture concentration	After 1 hour: Weight	Moisture	Moisture loss	Corrected weight of moisture	$\frac{D}{S}\times\frac{u_1+u_2}{u_1-u_2}$	After 3 hours: Weight	Moisture	Moisture loss	Corrected weight of moisture	$\frac{D}{S}\times\frac{u_1+u_2}{u_1-u_2}$
1	·330	9·79	24·84	16·836	17·247	·411	·0165	17·563	·727	·023	·732	·816	17·754	·918	·053	·926	·704
	·340	9·74	25·33	15·209	19·100	3·891	·1536	18·761	3·552		3·570		18·540	3·331		3·376	
2	·329	9·75	24·56	16·670	17·070	·400	·0163	17·390	·720	·013	·721	·812	17·584	·914	·046	·921	·694
	·334	9·74	24·88	14·988	18·785	3·797	·1526	18·452	3·464		3·476		18·225	3·237		3·276	
3	·325	9·79	23·71	14·632	14·920	·288	·0121	15·273	·641	·010	·642	·791	15·132	·850	·039	·856	·664
	·325	9·74	24·22	14·174	17·845	3·671	·1516	17·482	3·308		3·317		17·244	3·070		3·103	
4	·325	9·77	23·61	14·557	14·898	·341	·0144	15·227	·670	·013	·671	·798	15·432	·875	·030	·880	·670
	·324	9·73	24·08	14·072	17·675	3·603	·1499	17·333	3·261		3·273		17·111	3·039		3·064	
5	·308	9·77	23·09	15·700	16·043	·343	·0146	16·331	·631	·010	·632	·821	16·530	·830	·036	·835	·695
	·314	9·76	23·49	14·922	18·491	3·561	·1519	18·193	3·271		3·280		17·968	3·046		3·077	
6	·309	9·80	23·31	15·415	15·790	·375	·0165	16·061	·646	·000	·646	·827	16·257	·842	·023	·845	·700
	·314	9·78	23·59	14·818	18·331	3·513	·1489	18·060	3·242		3·242		17·841	3·023		3·043	
7	·330	9·78	24·79	16·818	17·241	·423	·0171	17·513	·695	·020	·697	·825	17·724	·906	·042	·912	·688
	·338	9·75	25·17	15·245	18·806	3·561	·1415	18·514	3·269		3·287		18·281	3·036		3·072	
8	·325	9·77	24·36	15·752	16·136	·384	·0157	16·430	·678	·001	·678	·816	16·632	·880	·016	·882	·688
	·338	9·71	25·02	15·240	18·815	3·575	·1429	18·520	3·280		3·281		18·303	3·063		3·077	
9	·318	9·75	23·93	14·575	15·013	·438	·0183	15·303	·728	·035	·732	·803	15·472	·897	·071	·908	·684
	·324	9·74	24·14	13·463	16·880	3·417	·1416	16·555	3·092		3·123		16·350	2·887		2·947	
10	·317	9·81	23·96	14·332	14·741	·409	·0171	15·031	·699	·025	·702	·808	15·213	·851	·049	·888	·686
	·328	9·74	24·44	14·090	17·545	3·455	·1414	17·230	3·140		3·162		17·024	2·934		2·975	
11	·323	9·77	24·21	15·566	15·971	·405	·0167	—	—	—	—	—	16·483	·917	·051	·925	·685
	·328	9·75	24·48	14·961	18·665	3·704	·1513	—	—		—		18·102	3·141		3·184	
12	·322	9·75	24·04	14·155	14·532	·377	·0157	14·822	·667	·027	·670	·821	15·002	·847	·057	·855	·709
	·325	9·73	24·16	14·694	18·352	3·658	·1515	18·035	3·341		3·365		17·825	3·131		3·180	

Table II (*continued*).

Exp.	Thickness (cm.)	Diameter (cm.)	Volume (c.c.)	Weight (gm.) Oven dry	Weight (gm.) Initial	Initial moisture	Initial moisture concentration	After 5 hours: Weight	Moisture	Moisture loss	Corrected weight of moisture	After 5 hours: $\frac{D}{S}\times\frac{u_1+u_2}{u_1-u_2}$	After 24 hours: Weight	Moisture	Moisture loss	Corrected weight of moisture	After 24 hours: $\frac{D}{S}\times\frac{u_1+u_2}{u_1-u_2}$
1	·330	9·79	24·84	16·836	17·247	·411	·0165	17·873	1·037	·084	1·052	·632	18·397	1·516	·138	1·609	·311
	·340	9·74	25·33	15·209	19·100	3·891	·1536	18·390	3·181		3·250		17·812	2·603		2·693	
2	·329	9·75	24·56	16·670	17·070	·400	·0163	17·713	1·043	·071	1·056	·615	18·210	1·549	·129	1·592	·299
	·334	9·74	24·88	14·988	18·785	3·797	·1526	18·071	3·083		3·141		17·507	2·519		2·605	
3	·325	9·79	23·71	14·632	14·920	·288	·0121	15·610	·978	·070	·992	·584	16·107	1·475	·124	1·519	·272
	·325	9·74	24·22	14·174	17·845	3·671	·1516	17·085	2·911		2·967		16·534	2·360		2·440	
4	·325	9·77	23·61	14·557	14·898	·341	·0144	15·567	1·010	·050	1·020	·584	16·051	1·494	·099	1·528	·272
	·324	9·73	24·08	14·072	17·675	3·603	·1499	16·956	2·884		2·924		16·423	2·351		2·416	
5	·308	9·77	23·09	15·700	16·043	·343	·0146	16·657	·959	·063	·969	·612	17·161	1·461	·117	1·503	·281
	·314	9·76	23·49	14·922	18·491	3·561	·1519	17·814	2·892		2·943		17·256	2·334		2·409	
6	·309	9·80	23·31	15·415	15·790	·375	·0165	16·378	·963	·047	·972	·620	18·866	1·451	·100	1·485	·293
	·314	9·78	23·59	14·818	18·331	3·513	·1489	17·696	2·878		2·916		17·155	2·337		2·403	
7	·330	9·78	24·79	16·818	17·241	·423	·0171	17·843	1·025	·071	1·038	·608	18·330	1·512	·116	1·551	·281
	·338	9·75	25·17	15·245	18·806	3·561	·1415	18·133	2·888		2·946		17·601	2·356		2·433	
8	·325	9·77	24·36	15·752	16·136	·384	·0157	16·752	1·000	·029	1·005	·611	17·232	1·480	·075	1·505	·297
	·338	9·71	25·02	15·240	18·815	3·575	·1429	18·170	2·930		2·954		17·644	2·404		2·454	
9	·318	9·75	23·93	14·575	15·013	·438	·0183	15·574	·999	·107	1·020	·609	16·015	1·440	·192	1·507	·282
	·324	9·74	24·14	13·463	16·880	3·417	·1416	16·212	2·749		2·835		15·686	2·225		2·348	
10	·317	9·81	23·96	14·332	14·741	·409	·0171	15·317	·965	·075	·999	·613	15·771	1·439	·154	1·492	·289
	·328	9·74	24·44	14·090	17·545	3·455	·1414	16·894	2·804		2·865		16·361	2·271		2·372	
11	·323	9·77	24·21	15·566	15·971	·405	·0167	16·608	1·042	·085	1·059	·604	17·116	1·550	·145	1·601	·275
	·328	9·75	24·48	14·961	18·665	3·704	·1513	17·943	2·982		3·050		17·375	2·414		2·508	
12	·322	9·75	24·04	14·155	14·532	·377	·0157	15·118	·963	·090	·980	·632	15·613	1·458	·151	1·509	·310
	·325	9·73	24·16	14·694	18·352	3·658	·1515	17·676	2·982		3·055		17·120	2·426		2·526	

Table III.

Mean values obtained for $\frac{D}{\bar{S}} \times \frac{u_1 + u_2}{u_1 - u_2}$ *from experiments of different duration for the two thicknesses of material used, together with the probable errors for each series.*

1/16th inch material. Mean thickness of a pair of discs 0·323 cm.

Duration of experiment (hours)	No. of experiments	$\frac{D}{\bar{S}} \times \frac{u_1+u_2}{u_1-u_2}$	Probable error of mean	Probable error of one observation
1	29	·646	·0046	·025
2	16	·534	·0070	·029
3	12	·436	·0055	·019
4	14	·364	·0054	·020

1/8th inch material. Mean thickness of a pair of discs 0·650 cm.

Duration of experiment (hours)	No. of experiments	$\frac{D}{\bar{S}} \times \frac{u_1+u_2}{u_1-u_2}$	Probable error of mean	Probable error of one observation
1	48	·814	·0019	·013
2	32	·724	·0025	·014
3	31	·683	·0021	·012
4	47	·610	·0024	·017
5	30	·600	·0025	·014
6	30	·550	·0038	·021
24	38	·281	·0026	·016

Table IV.

Values of $\frac{D}{\bar{S}} \times \frac{u_1 + u_2}{u_1 - u_2}$ *taken from the curves in Figs.* 1 *and* 2 *on which the calculation of the coefficient of moisture conductivity* k *and its probable error was based.*

1/16th inch material.

Time (hours)	$\frac{D}{\bar{S}} \times \frac{u_1+u_2}{u_1-u_2}$	Value taken for P.E. of mean of $\frac{D}{\bar{S}} \times \frac{u_1+u_2}{u_1-u_2}$	k	Probable error of k
1	·650	±·005	·00250	±·00007≡2·8 %
2	·532	±·008	·00224	±·00008≡3·6 %
3	·435	±·006	·00219	±·00005≡2·3 %
4	·361	±·005	·00214	±·00004≡1·9 %

1/8th inch material.

Time (hours)	$\frac{D}{\bar{S}} \times \frac{u_1+u_2}{u_1-u_2}$	Value taken for P.E. of mean of $\frac{D}{\bar{S}} \times \frac{u_1+u_2}{u_1-u_2}$	k	Probable error of k
1	·805	±·002	·00315	±·00012≡3·8 %
2	·728	±·003	·00308	±·00007≡2·3 %
3	·672	±·002	·00298	±·00006≡2·0 %
4	·630	±·003	·00284	±·00004≡1·4 %
5	·590	±·003	·00279	±·00004≡1·4 %
6	·552	±·004	·00277	±·00002≡0·7 %
24	·280	±·008	·001895	±·000025≡1·3 %

Table V.

Some theoretical values of $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ *for the two thicknesses (mean values) of material used in the experiments for two different values of the coefficient of moisture conductivity.*

When the combined thickness of the pair of discs is 0·650 cm.

Time (hours)	Log. time	Value of $\frac{D}{S} \times \frac{u_1+u_2}{u_1-u_2}$ when k=·00320	when k=·00185
0·5	−0·3010	·8616	·8939
1	0·0	·8039	·8502
2	+0·3010	·7223	·7889
3	0·4771	·6600	·7416
4	0·6021	·6073	·7013
5	0·6990	·5610	·6661
6	0·7782	·5193	·6342
10	1·0000	·3841	·5281
14	1·1461	·2847	·4428
19	1·2788	·1959	·3567
24	1·3802	·1348	·2873

When the combined thickness of the pair of discs is 0·323 cm.

Time (hours)	Log. time	Value of $\frac{D}{S} \times \frac{u_1+u_2}{u_1-u_2}$ when k=·00320	when k=·00185
0·5	−0·3010	·7206	·7874
1	0·0	·6047	·6995
2	+0·3010	·4431	·5738
3	0·4771	·3271	·4801
4	0·6021	·2416	·4026
5	0·6990	·1785	·3378
6	0·7782	·1319	·2835
10	1·0000	·0393	·1408
14	1·1461	·0117	·0699
19	1·2788	·0026	·0291
24	1·3802	·0006	·0121

Table VI.

Mean values of $\frac{D}{S} \times \frac{u_1 + u_2}{u_1 - u_2}$ *for two series of seven experiments with 1/8th inch material for three repetitions.*

(*a*) The one, three, five, twenty-four hour series.

	1 hour	3 hours	5 hours	24 hours
1st time	·807	·677	·593	—
2nd „	·811	·688	·608	·289
3rd „	·801	·677	·589	·288

(*b*) The two, four, six, twenty-four hour series.

	2 hours	4 hours	6 hours	24 hours
1st time	·728	·624	·560	—
2nd „	·733	·634	·563	·276
3rd „	·730	·647	·563	·294

THE MEMBERS OF THE GENUS *AGROSTIS* IMPORTANT IN THE SEED TRADE, WITH SPECIAL REFERENCE TO THE NOMENCLATURE

By ALEXANDER NELSON, B.Sc., N.D.A.

While none of the members of the genus *Agrostis* are at present important in agriculture in Great Britain, the fact that the trade in their seeds is largely international and, to a great extent, passes through this country, renders some knowledge of the international nomenclature of peculiar importance to seedsmen.

It may be stated at the outset that not only are the national literatures referring to the common names of the members of the genus in the different countries often vague and self-contradictory, but the botanical naming of the various species is in a chaotic state. All genera with cultivated members appear to be, more or less, in a similar state; the greater the number of species or varieties cultivated, the deeper the chaos. Such is the position that one doubts if the problem will yield to treatment along traditional lines and seems to call for co-ordinated effort, systematic and genetic, on the part of those dealing with cultivated types in the manner suggested by Drummond (6).

From the seed-trade point of view there may be taken to be three broad classes of *Agrostis*:

(i) Those seeds which are dealt in and sold for general purposes, mostly agricultural, *i.e.* for the production of hay and/or pasture.

(ii) Those for which there is a special use and for which a special provision is made, usually for the production of lawns.

(iii) Seeds which occur promiscuously as impurities, either in samples of seed of other genera or in samples of *Agrostis* sold under a definite name and where they can be recognised by the analyst.

Having noted these classes, it is necessary to enquire briefly what characters of a botanical nature would relegate any one plant to any class and render it, on the one hand, valuable to agriculture and/or horticulture, or, on the other hand, deleterious. In agriculture, all that is required is that the plant produce hay or pasture superior, or, at least, equal to any other plant which can be grown on the soil and

under the conditions in question. Also, that it persist but be easily eradicated by ordinary cultural methods when it has fulfilled the purpose for which it was sown and its removal is desired. Apart from those species which are grown purely for their ornamental value and which need not be further considered here, the horticulturist employs members of the genus in the formation of lawns. In this connection the character most insisted on is that the plant be a surface creeper and bind the other constituents of the lawn well together while producing fine, well-wearing verdure. Though the question of easy eradication does not arise prominently in connection with lawn formation, it is interesting to note that, though for different reasons, the horticulturist and the agriculturist both desire a surface creeper; the horticulturist, because a subsurface creeper does not bind the constituents of his lawn well; the agriculturist, because it is a difficult type to eradicate, and also because a subsurface creeper stores most of the surplus food below the soil, out of reach of grazing animals. It is clear, then, that in general agriculture or horticulture the desirable type cannot be a subsurface creeper. Thus are arrived at two apparently sharply divided classes founded on morphological features:

(i) Surface creepers producing desirable leafage.

(ii) Subsurface creeping types and those which do not produce desirable leafage.

From these criteria of what is good and what is bad, it is useful to enquire as to how far the seed trade can go in supplying seed likely to produce plants of a desirable type. Treating at the moment, only of the purity as defined by the seed analyst, it is generally admitted that few, if any, samples are pure to any one major type or species (Percival (12), Armstrong (1), Stebler (18)). The important point, however, is, not the question of the analytical purity, but the question of purity as defined by the geneticist. The present writer has been unable to trace any work bearing directly on this aspect, but some writers have offered significant clues.

E. Bruce Levy (4), states, "The cause of variation within and between the species" (*i.e.* of *Agrostis*) "is extremely difficult to account for, but it would appear that hybridisation played an important part, for it is in areas where the three species" (three aggregates, see below) "may be found together that one notes the greatest variation and often the most puzzling of forms. Again, where the habitat is such as to favour only the one species, the growth-form and character of that species become more constant. Also, there is affinity between the aggregate species. This

affinity asserts itself not so much by a general similarity in the plants, but rather by a manifestation of special features, such as the appearance of the long rhizomes in otherwise short root stock forms, or by the ready stolon development of otherwise non-stolon erect forms. These variations are distinct from habitat variations, which at times are extremely marked—so much so that to define the vegetative growth-form of the species botanically is almost impossible."

Ascherson and Graebner (2) mention a number of hybrids and suggest that one recognised species (*Agrostis nigra*) is a hybrid arising out of *Agrostis alba* × *Agrostis vulgaris*. M'Alpine (10), p. 77, states: "This genus" (*Agrostis*) "is extremely liable to variation and accordingly is very difficult to define." The same writer (11), writing of Common Bent, which he refers to *Agrostis vulgaris*, states: "In fact, Common Bent adapts its mode of growth to those circumstances in which it finds itself. If the water is on the surface, it is a surface creeper; if the water is deep underground, it becomes a deep underground creeper." Many other writers emphasise this variability of the members of the genus, but it is nowhere clear that more than observational evidence of plants in the wild is offered. Sinclair (16), however, in a statement further discussed below, records that he found in controlled cultivated plants taken from the wild, certain types which were valuable *constant* surface creepers on ordinary loam and which, in one case at least, he refers to *Agrostis stolonifera* var. *latifolia*. From consideration of these citations, it would appear that some at least of the reputed species of *Agrostis* are interfertile and that many of the plants are of hybrid genetical constitution. Also, it would appear probable that many of them are capable of carrying factors for the production of certain major botanical characters, the expression of which may be suppressed by the environment. This possible alteration of such a character as stolon formation or alteration of the plane in which the stolons are produced, by environmental factors alone, opens up a very serious question for those workers who are attempting to breed up desirable strains of *Agrostis*, and indicates that before such strains are introduced into commerce they will require to be tested over a very large and varying range of situations. Despite all this variation, it is probable that the trade in seeds of members of the genus is likely to continue in the future along lines much as at present, and therefore it is of value to consider the various names, botanical and commercial, connected with the business, and equate them one to the other. In this connection the confusion in nomenclature which exists in most countries is, as has already been mentioned, further intensified

by the fact that the trade is largely international. There is a current of trade in one kind from Central and South Europe, sometimes via this country and sometimes direct to America. There is a current of trade from America to this country and Europe, while New Zealand is exporting to America and Europe generally. The procedure to be adopted here is to sift out each name in each country concerned and then, as far as possible, equate the results.

The first trade name which calls for attention is that of the genus itself: quite a number of samples change hands, described simply as *Agrostis*. There is little or no difficulty here, because, obviously, anyone purchasing under this description must be satisfied if the seeds (within the declared analysis) produce forms of *Agrostis*, no matter how mixed the types. "Bent Grass" (unqualified) may be taken to be synonymous with *Agrostis* in this connection.

Perhaps the most widely used and most ambiguous term is "Fiorin." In general commerce now, particularly in the home trade, this too, is taken to mean an indeterminate mixture of species of *Agrostis*. Trouble soon arises, however, when, as often happens, samples are described as "True" or "Genuine" Fiorin. What is implied by "true" or "genuine"?

Dr William Richardson, of Moy, Ireland, first introduced the name "Fiorin" about the year 1807 and applied it to a very luxuriant stoloniferous grass admirably suited to agricultural uses. It is probable that the type was not too well defined, being propagated mostly by vegetative means, though it seems clear that it was included under *Agrostis stolonifera*. Great confusion has arisen from the use of this name: the type is difficult to define and the name has arisen on more than one occasion. Sinclair(16), states: "That our *Agrostis stolonifera* is the *Agrostis alba* of Linnaeus is clearly proved by Sir James Edward Smith in his 'English Flora.' The error seems to have originated in Withering."

Percival(12) describes Fiorin under *Agrostis alba* L., and notes that the structure and habit are liable to vary. Armstrong(1) describes it under *Agrostis alba* L. var. *stolonifera* and emphasises the fact that it throws out *creeping surface stems*: later he describes Black Bent Grass, *Agrostis alba* var. *repens* = *Agrostis nigra* Withering, as having *stout underground rkizomes*. The important point here is the recognition of the two forms or varieties, and the splitting of the species. It is noteworthy that, of the two varieties, one is outstandingly valuable and the other the reverse. The same writer later describes another species, *Agrostis vulgaris* Withering, and suggests that it is simply a small variety of *Agrostis alba* L. Marshall Ward(9) describes Fiorin under *Agrostis*

stolonifera, but suggests that it, along with *Agrostis vulgaris* Withering, and possibly *Agrostis canina*, are mere varieties of *Agrostis alba* L. Notwithstanding this confusion, interfertility and variability between the species it seems clear that the *stolonifera* variety of *Agrostis alba* L. described by Armstrong actually exists in a constant state, being synonymous with Sinclair's *Agrostis stolonifera* var. *latifolia*, and is, moreover, the "ideal" Fiorin.

Somewhat the same sort of position is found on consideration of the main European literature. Hein (8) notes that most of the trade samples are mixtures, the resulting plants being difficult to differentiate, and he says: "There is the stolon pushing variety of *Agrostis alba*, also the creeping variety of *Agrostis vulgaris*, and the more common variety of stem grass, which is a combination of these." Later he expresses the opinion that only the wet land forms, especially the stoloniferous variety of *Agrostis alba* L. can be called "*Agrostis stolonifera*" "Fiorin." (Nach meiner Ansicht kann indess nur die auf nassem Boden besonders vorkommende Abart von *A. alba* L., die species β[1] mit Recht "*Agrostis stolonifera*" und "Fioringras" genannt werden.) Hein quotes Courtin (Kulturgräser und Futterkräuter), who refers Fiorin grass to *Agrostis alba* var. *stolonifera*.

Stebler (18) describes Fiorin or stoloniferous Bent Grass as *Agrostis stolonifera* L. or *Agrostis alba* Schrad. and mentions that the grass throws out long stolons which may lie *either above or beneath* the ground. He differentiates the grass from *Agrostis vulgaris* Withering. Ascherson and Graebner (2) show quite clearly that from the start the specific name *stolonifera* has been a vague one to which many types have been referred. These authors, under the name *Agrostis stolonifera*, form a composite species which includes *Agrostis alba*, *Agrostis nigra* and *Agrostis verticillata*. Under *Agrostis alba* the common name "Fiorin" is given and the statement is made that this grass is almost *exclusively surface creeping*, very rarely having creepers below ground. *Agrostis verticillata* is described as being almost exclusively *a subsurface creeper*. Under *Agrostis nigra* no indication is given as to the plane of the creeping shoots and the authors state that the systematic significance of this species seems doubtful and suggest that it represents a fertile hybrid from *Agrostis alba* × *Agrostis vulgaris*.

Owing to the importance given to species of *Agrostis* in America, where they are largely used in the formation of golf-course greens, etc., the literature is extensive, and it is found that writers have ceased to

[1] Species β = *Agrostis alba* L. β *stolonifera*. E. Meyer, English Fiorin, Bent Grass.

use the Linnaean names and have adopted those of other authorities. Piper and Hillman (14) refer Fiorin to *Agrostis maritima* Lamark; *Agrostis stolonifera* var. *latifolia* Sinclair, and note that this is Richardson's grass referred to *Agrostis stolonifera* by many writers.

Hitchcock (7) examined the specimens in the Linnaean collection, particularly those marked in the handwriting of Linnaeus himself, and from his description it seems clear that it is the stoloniferous form of *Agrostis alba* L. which is desirable in agriculture and horticulture.

Beale (3) under *Agrostis alba* L. mentions Creeping or Marsh Bent, Fiorin, White Bent or White Top, thought by some to be *Agrostis stolonifera* L. Beale suggests that this is a variety of "Red-top" or *vice versa* (see "Redtop" below).

Clarke and Malte (5) adopt the view that Fiorin and Redtop are synonymous, because under Redtop = *Agrostis stolonifera* they mention such other names as *Agrostis alba* L. Fiorin, Creeping Bent, etc.

The only other country at present handling Bent grasses in the International market is New Zealand. E. Bruce Levy (4) deals with the New Zealand forms and reduces them to three large aggregates, the first of which he designates Redtop, *Agrostis palustris*, *Agrostis alba* of most botanists, Fiorin of the seed trade, *Agrostis stolonifera* of some seedsman's catalogues, "twitch." In his third aggregate, the same writer includes Creeping Bent (*Agrostis stolonifera*), Carpet Bent of American greenkeepers, *Agrostis depressa* and *Agrostis maritima* of some American botanists. He states that this is the grass commercialised by Richardson under the name Fiorin. The outstanding feature of the grass described under this third aggregate by Bruce Levy is its long overground stems (stolons).

Regarding all these citations referring to Fiorin, the following facts emerge: "Fiorin" is a luxuriant surface creeping grass which, in the earlier days of its history, was referred, along with many other types, to *Agrostis stolonifera* L. The type of *Agrostis stolonifera* has been from the beginning only vaguely defined and its general use might be discontinued, as it has led and is leading to confusion. Not only has this association with a vague though seeming specific name directly confused matters, but it has led in large part to a progressive debasement of the name Fiorin. In the beginning, the then *true* Fiorin was justifiably ranked as an excellent pasture grass, yielding as it does good grazing at a time of the year when other grasses are dormant. Either from the fact that useless and injurious types (underground creepers, etc.) were classified along with it in *Agrostis stolonifera* L. or from deliberate or

ignorant substitution, the specificity of the name Fiorin was lost and the grass quickly fell into disfavour. When, however, the true surface creeping type was clearly recognised and its constancy established, the position became clearer and the possibility of a genuine Fiorin emerged. The only known commercial source of such a grass is South Germany, from whence come samples which undoubtedly do contain some genuine Fiorin (about 1 per cent. according to Bruce Levy). In course of time these samples became better known, commanded a higher price and were named Genuine South German *Agrostis*, or latterly and more briefly, Genuine Fiorin. Hence the appellation Genuine has ceased to have any real significance. It is perhaps worthy of note that samples of this type received direct from Germany usually bear, alone or in addition, the name *Agrostis stolonifera*. This term, if used, as by Ascherson and Graebner, and to some extent by other botanists, to indicate a mixture of stoloniferous forms including True Fiorin, then the term would appear to be, in some degree, justified. It emerges, however, that there is at present in commerce no such thing as pure or semi-pure Genuine Fiorin, though its isolation and introduction should be welcomed and would probably prove profitable to the introducers. Any precise statement as to which botanical species Genuine Fiorin is to be referred does not come within the scope of this paper, but it seems abundantly clear that the grasses described by Sinclair under *Agrostis stolonifera* var. 1 *latifolia* and by Armstrong as *Agrostis alba* L. var. *stolonifera*, are very near, if not the ideal type. However, for practical purposes the filing of botanical specimens and description along with the naming may safely be left to the introducer of a non-varying, permanent strain.

The next trade name which falls to be considered is one not commonly known or used in British Agriculture, but widely used in the seed trade and is of American origin: this is "Redtop."

Hitchcock (7), Piper (13), Spillman (17) and others refer this grass to *Agrostis alba* L. According to Hillman who, along with Piper (14), refers it to *Agrostis palustris* Huds. (*Agrostis alba* of most botanists), it includes relatively few types. Bruce Levy names one of his aggregates "Redtop" and agrees with Piper and Hillman in referring the grass to *Agrostis palustris* Huds. Redtop would appear to be nearly synonymous with the commercial Fiorin of the British seed trade, differing perhaps in that it is comprised of fewer types.

"Rhode Island Bent Grass" another American trade name becoming known in this country refers, according to Piper and Hillman, to *Agrostis*

tenuis Sibth., and is identical with *Agrostis vulgaris* Withering. This grass is recommended for lawn formation by Hillman, who notes that samples are likely to contain "Redtop" amongst other types.

Bruce Levy names his second aggregate "Brown top" or Colonial Bent referred to *Agrostis tenuis*, the *Agrostis vulgaris* of most botanists. He agrees with Piper and Hillman that the New Zealand samples passing as Colonial Bent are identical with American Rhode Island Bent. A number of samples have been offered on the British market under the name "Waipu Brown Top." This is a name used only by Auckland seed merchants and growers and refers to Colonial Bent as noted above.

Confusion between Brown Top, as defined above, and the Brown Bent of British literature must be guarded against. Brown Bent is referred by British and American writers to *Agrostis canina* L. It is sometimes called Velvet Bent in America and strongly recommended for lawn production.

There are various other names current in the British seed trade such as Kentish Bent and so on, but these are all more or less mixtures of species of *Agrostis*. The Carpet Bent so much prized by American greenkeepers seems to be "Genuine Fiorin" as defined in this paper.

Summary.

1. The paper deals with the members of the genus *Agrostis* occurring in the international seed trade; current names are enumerated and, as far as possible, related to each other.

2. The following relationships are established:

Trade Designation	Botanical Equivalent
Bent Grass (unqualified) *Agrostis* (unqualified) Fiorin (unqualified)	Indeterminate mixtures of species and/or forms of *Agrostis*
Redtop or Red Top	Do., but probably comprising fewer types
"True" Fiorin "Genuine" Fiorin ? Carpet Bent	Should be the "Fiorin" of Richardson, *i.e. Agrostis stolonifera* var. 1 *latifolia* of Sinclair or *Agrostis alba* L. var. *stolonifera* of Armstrong. No pure strains in commerce though certain samples may contain a small percentage
Rhode Island Bent Brown Top Colonial Bent Waipu Brown Top	*Agrostis vulgaris* With.
Brown Bent Velvet Bent	*Agrostis canina* L.

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STUDIES ON THE AETIOLOGY OF SUGAR-CANE FROGHOPPER BLIGHT IN TRINIDAD

I. INTRODUCTION AND GENERAL SURVEY

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(With 4 Text-figures.)

Froghopper "blight" of sugar-cane is confined to Central and South America, but it would seem to have attracted most attention in Trinidad, where the present investigations have been conducted. Superficially the "blight" somewhat resembles Sereh disease of the East Indies, the similar symptoms being probably due to similar effects upon the metabolism of the cane plant. Sereh disease is now believed to be due to infection with *Bacterium herbicola aureum*, but in the case of froghopper "blight" damage to the leaves is the result of the sucking of a Cercopid, *Monecphora* (*Tomaspis*) *saccharina* Dist. The effect of "blight" upon a canefield has often been likened to a scorching by fire and certainly the leaves of attacked canes die and dry up in the manner in which one would expect if scorched.

J. H. Hart, in 1890 (9) was the first to attribute "blight" definitely to the cane froghopper and he also noticed the contemporary presence of root fungi in the badly attacked fields. Since Hart's time the froghopper has received attention at the hands of several specialists (*vide* Williams (29)) who have contributed to our knowledge of the problem. The most recent work on the subject is the report of C. B. Williams (29), which besides adding an enormous number of observations to previous ones gives an exhaustive summary of earlier work, including an excellent bibliography. To Williams' fine work I must therefore refer for many details and supplementary evidence in connection with the present studies.

After reading Williams' report it is quite evident from the apparently conflicting circumstances associated with the "blight" that an enormous number of factors are involved no one of which can be isolated as the principal cause. In the main, however, prevalence of the disease seems

to be governed by environmental conditions some of which are capable of alteration by agricultural methods and others over which we have as yet no control. Nowell and Williams, especially, but other investigators as well, have emphasised the necessity for improvements in agricultural practice and with their opinion anyone who has studied the problem cannot fail to agree, but still the inner reasons and the exact influence of such methods upon the "blight" are but little understood. There is an immense field of research awaiting any investigator in meteorology, agricultural engineering, soil science and plant or insect physiology, to mention only a few of the many varied aspects. The present enquiry was commenced in the hope of clarifying the interrelationships of insect and plant, but it has hardly done more than indicate a few of the many lines along which further work must be prosecuted by specialists in various sphéres. The question is too vast for a single investigator.

Williams(29), p. 96, groups the factors influencing prevalence of "blight" under four headings:

(1) Physical environment; *e.g.* rainfall, temperature, soil and contour of land, over which man has little or no control.

(2) Agricultural processes; *e.g.* drainage, tillage, rotation, manuring, etc.

(3) Characteristics of cane; there being variations in resistance to injury.

(4) Direct effect of number of froghoppers present and the effect of fungal and other diseases in weakening resistance of the cane and preventing recovery.

Details of all these groups of factors are given and it would be impossible to condense them here without prejudice. Again, the views of earlier workers, which are given due consideration by Williams cannot be compared or evaluated in the present paper without occupying much space. References to all will be found in Williams' bibliography.

In the first of this series of studies it is proposed to give a general account of the interrelationships of sugar-cane plant and the froghopper, more particularly the adult insect, and from such central viewpoint the problem will be surveyed in a few of its aspects. It is hoped thereby to offer explanations of some of the previously observed although but vaguely understood phenomena and to attempt coordination.

There are obviously two important sides to the question of froghopper blight which require elucidation; (1) the factors controlling the numbers of the froghoppers themselves, and (2) the conditions in-

fluencing resistance of the canes to and recovery from attack. Both may be interdependent and evidently often are, but it is to the second aspect almost entirely that attention will be devoted in the present paper.

Bionomics of Froghopper.

The Trinidad Froghopper, *Monecphora* (*Tomaspis*) *saccharina* Distant, is a typical Cercopid, averaging in length about 8 mm. The general colour is dark brown with two narrow transverse bands on the forewings of a pale whitish-ochreous colour. The bases of the forewings are also often pale in colour, especially along the inner margin at the base.

Males and females occur in the field in fairly equal numbers. Males principally are attracted to lights. The insects are inactive during the daytime and remain, head uppermost, in the hearts and leaf axils of the canes. Williams (29), p. 57, states that "apparently little or no feeding is done in this position." Personally, I have frequently observed froghoppers feeding thus throughout the entire day, but as the specimens positively seen were in a shaded cage under experimental conditions and not in the open the observation is of less value. In the field I feel less confident of the accuracy of my observations. It is not easy to look into the hearts of the canes without disturbing the insects, but from the frequent accumulation of faecal water I think that they continue feeding. After sunset the froghoppers become active and, especially the males, fly. Feeding and pairing occurs. An hour or two after sunrise the hearts and axils of the leaves are again resorted to.

From 50 to 100 yellowish eggs (·75 × ·25 mm.) are laid by each female, generally in the dead ensheathing leaf-bases of the canes or grass. The female uses her ovipositor to slit the plant tissues for the insertion of each egg. Eggs may hatch in about two weeks in the wet season or they may aestivate for a period of some months during the dry season. Eggs are readily laid by females in captivity in damp filter paper and can thus be easily studied.

The nymph is of usual Cercopid form. Kershaw (12) states that there are five moults (six including the embryonic moult which occurs on hatching) and therefore five nymphal instars. Williams (29) could only trace four instars. I cannot decide definitely.

Nymphs live generally upon the roots of the cane and other grasses and suck the sap from the roots fairly continuously. Correlated with the habit of more continuous sucking one finds that the salivary glands of the nymph are less developed than those of the adult and it is to be expected that the drain upon the plant juices by the nymph is slower

and requires less salivary action than that of the adult. The leaves of small cane plants were found to become yellow and unhealthy with a number of froghopper nymphs feeding upon their roots, comparison being made with control plants free from froghopper nymphs, but such an experiment was hardly comparable with natural conditions. From field observations it has appeared that the adult phase is the more important and therefore I would devote attention in this first paper mainly to a consideration of the adult.

The waste fluid matter excreted by the nymph together with a mucin or mucinoid which Kershaw(14) states, as I believe correctly, to be secreted by the Malpighian tubes, is blown into a froth by means of the ventral abdominal cavity, formed by the overlapping sides of the abdomen, which is used as a bellows. Into the ventral abdominal cavity the abdominal spiracles also open and doubtless some respired air enters into the formation of bubbles. The action of bubble-blowing can easily be watched. The nymph pushes the tip of its abdomen through the froth to the outside air, takes a fresh supply of air into the abdominal cavity and then expels this air in the form of a uniform bubble from the tip of the abdomen wherever a bubble requires to be added to the froth mass. The tip of the abdomen is moved actively into all required positions.

The froth of the nymph is certainly a protection against desiccation and against insect enemies. It may possibly serve also as a reserve air supply and could be drawn upon by the nymph in time of flood and the like. It is an effective barrier against insecticides unless these can dissolve in and so penetrate the froth.

Nymphs feed upon the roots at various depths, these last being determined largely by two factors; (1) humidity, and (2) texture of the soil. I am of the opinion that the second factor is only of importance as it affects the first. In wet weather the nymphs appear at or near the surface of the ground and if flooded they will crawl up the stems of the canes or grasses and live temporarily in the leaf axils or even on the leaves and stems. (In tall jars in the laboratory they will establish themselves almost anywhere upon the leaves and stem, given a saturated atmosphere.) With decrease in soil moisture they are found at progressively greater depths and in the height of the dry season they can be obtained as far down as ten inches or a foot—at least on Uba cane. The closer the texture of the soil, with consequent less free interchange of soil gases, the more superficial, as a rule, is the habitat of the nymph; in well-broken soil it is generally deeper. Surface soil moisture, such as is furnished by mulches and matted cane trash, will also induce nymphs

to live nearer the surface. These are merely general rules; individuals vary. Especially when about to transform to the adult does the nymph approach the surface of the ground, usually forming there its last froth mass prior to emergence as an imago. Under favourable wet season conditions the life cycle is completed in about two months, sometimes less.

The dry season (January to May, approx.) is stated by Williams (29) and others to be passed in the egg stage, and this is undoubtedly true in the great majority of cases, nevertheless nymphs may continue in small numbers throughout the greater part of the dry season in open dry fields as well as in damp valleys. This point first suggested itself when it was found that a day or two after a good shower of rain in the dry season one or two adult froghoppers could be obtained in the course of an hour or so of sweeping, especially along drains, where previously none had been obtainable. A possible explanation was of course that the mature adults underground were merely awaiting sufficient rain to appear at the surface, or nymphs awaiting the final moult had been stimulated by the rain to emerge as adults. Cane stools in previously affected fields were examined and a few full-fed nymphs were found in February, April and May 1925, at a depth of from 6 to 10 inches underground. Mr G. A. Jones found nymphs similarly at Usine Ste. Madeleine in March. Adults were not found then. It seems therefore probable that the development of nymphs may be retarded or arrested when full fed in the dry season and these nymphs remain underground until the stimulus of rain induces them to emerge as adults.

In the present connection the following data may be of interest. They were obtained from Usine Ste. Madeleine, Williamsville Estate, in the dry season 1925.

Since January 4th no rain so much as an inch in a day had fallen. In the two weeks preceding the first real shower ·09 inch fell on March 18th and ·04 inch on March 21st. On March 26th 1·31 inches fell and next day ·60 inch. The rain on these two consecutive days induced the appearance of adult froghoppers. No rain fell again until April 1st with ·32 inch, April 3rd ·02 inch, April 4th ·05 inch, and April 7th ·09 inch. During the first week in April signs of attack upon the leaves of the canes were noticed in one small patch of land (100 × 50 yards) on which were growing Uba plant canes. The local nature of the attack may be difficult to explain—the manager considered it to be a bad patch of land—but the point now wished to illustrate is the appearance of adult froghoppers after a sufficient shower, and, be it borne in mind that the immediately subsequent weather was dry.

The next rain equal to an inch in a day occurred on June 4th with 1·04 inches, June 5th with 2·61, followed by another drier spell, viz. ·04 inch on June 8th, ·08 inch on June 10th, ·02 inch on June 11th, and ·06 inch on June 12th. On June 13th I visited the locality and the marks on the leaves of the canes were such as would have developed in from one to one and a half weeks. A few adult froghoppers were still present. It is quite obvious from these two outbreaks in the same locality that the froghoppers emerged almost immediately upon the stimulus of rain, and injury to the leaves assumed distinctness a week or two afterwards, with drier weather. The effect of dry weather is dealt with later in this paper.

Adults of *Monecphora saccharina* will feed upon almost any species of grass (Ord. Gramineae) and some sedges (Ord. Cyperaceae). I have also known them to feed upon *Canna edulis* in small numbers and to cause the appearance of yellow marks on the leaves of this plant. Other plants are sometimes sucked to a small extent. As a pest one associates the species particularly with sugar cane; very occasionally with maize.

The feeding of the adult seems to be more rapid than that of the numph and it will now be dealt with. Details of the salivary mechanism and that of the nymph must be left for consideration in another of the present series. It has already been briefly noted by Kershaw (13) but there are many additional points of interest requiring fuller description.

As in other Rhynchota, the mouthparts of the adult froghopper consist of styliform mandibles and maxillae which are ensheathed by a four-jointed labium, the latter being furnished at its distal extremity with small sensory hairs. The maxillae are simple and pointed, grooved on their inner sides and together forming two tubes, a dorsal suction canal and a ventral salivary ejector canal. The mandibles lie on either side of the combined maxillae; they are also grooved internally and pointed apically but in addition they are serrate near their apices. Activating the stylet mouthparts are muscles contained within the head capsule.

The juices of the plant are sucked up by means of a large pharyngeal pump within the head. This takes off sap from the dorsal maxillary canal at the point where the stylets diverge on entering the head capsule. The pharynx, or as it might be better termed, the buccal cavity, is at first narrow antero-posteriorly. On the roof of its first portion are sense organs which are no doubt gustatory in function. Immediately behind this tasting region is a valve with muscles attached, which, from its

structure would be most efficient in preventing regurgitation from the pharyngeal sucking bulb.

Ventrally to the pharyngeal pump is a powerful salivary pump which forces saliva along a spout opening just in front of (ventrally to) the gustatory region of the buccal cavity. Thus saliva is forced between the stylets at their point of concurrence and into the ventral salivary ejector canal. The salivary pump consists of a chitinous piston working within a bulb, into the floor of which the united ducts of the salivary glands open. To the piston are attached muscles. Retraction of the piston produces a negative pressure within the salivary bulb, causes closure of the spout valve and withdraws saliva from the salivary glands. Return of the piston into the salivary bulb forces saliva from the bulb into the spout and down the stylet salivary ejector canal.

The salivary glands have already been described by Kershaw (14). They consist of a small head gland and a larger thoracic gland which possesses twelve long diverticula. The main body of the thoracic gland is peculiar and interesting in structure. It will be described elsewhere.

Feeding occurs as follows. The proboscis, which, owing to flexure of the head comes to project from rather well back, almost between the coxae of the first pair of legs, feels about for a suitable spot on the cane leaf. The position is no doubt tested by the sense organs at the tip of the labium and a spot near a vein is generally selected. As the veins in the leaf are very close together this selection cannot be difficult and probably the froghopper really only has to select a place as free as possible from siliceous thickening. Having chosen a feeding spot the serrate mandibles are driven into the leaf. This sometimes occasions a slight twisting or lateral movement of the body, especially in starved, weak insects. The smoothly pointed maxillae follow the mandibles, combined as a central column. These can be extended beyond the apices of the mandibles and they contain the sucking channels. The serrated mandibles seem to break through the hard cuticle of the leaf and then remain embedded firmly while the maxillae pierce other deeper tissues and conduct back their fluid contents.

Immediately and while the stylets are still boring into the leaf there is an exudation of saliva which can be seen around the stylets at the surface of the leaf. This saliva is not large in quantity but it can be distinctly observed under a low power microscope. Within a minute usually the small amount of saliva is seen to have been reabsorbed, indicating that sucking has commenced. In a further period of from one to three minutes another exudation of saliva occurs and this again

is reabsorbed within a quarter of a minute or less after its appearance. The process of exudation and reabsorption of saliva then repeats regularly every minute or so. Such is a fairly typical case. Times vary.

It is seen that salivary expulsion usually precedes sucking. The saliva may serve as a stylet lubricant during the process of boring the leaf; its main function, however, is that of an extra-oral digestive, as will be seen later. In some cases salivary exudation is not conspicuous: subsequent examination of the leaf shows this to be correlated with deeper and more destructive puncture, generally inclusive of the xylem vessels. It is also frequently associated with more rapid excretion of faecal water and generally with more robust and healthy insects. The operation first described can always be observed in an insect which has been starved all day and is consequently hungry and somewhat weakened. At first, starving the insects was done on purpose to observe feeding under the microscope. Vigorous and unstarved froghoppers may be rather restless and less tractable for observation.

Feeding times and methods vary. If a suitable position has not been found the froghopper will leave the place within a minute or so and puncture elsewhere nearby. A suitable position is probably determined not only by the amount of fluid withdrawable and the facility of sucking, but also maybe by the taste experienced at the gustatory patch on the roof of the mouth. On one occasion a froghopper was noted to make about 100 punctures in a small area 2 cm. long by 3 mm. broad. In this rather exceptional case the insect retained hold of the edge of the cane leaf with its legs of one side. It moved from side to side and also backwards during the several attempts at feeding. Other instances have been observed when satisfactory nutriment was obtained from one puncture during an hour or more.

Depth of puncture varies. Often the stylets penetrate to the epidermis of the opposite side of the leaf, at other times they go no deeper than the precincts of the vascular bundles. I have no evidence of preference for upper or lower sides of leaf. Under experimental conditions both sides have been attacked when the leaf has been vertically placed. Field observations are inconclusive.

On disturbance the adult froghopper frees its stylets quickly and although I have occasionally killed an insect with stylets *in situ* these could not be retained in passage through reagents for embedding and sectioning.

After withdrawal of the stylets a minute puncture can be seen and

around this generally a very small area with a whitish efflorescence which is the dried saliva.

During feeding small drops of almost colourless fluid are voided from the anus at the rate of one every two to five minutes. Six froghoppers allowed to feed on a normal, full-sized cane leaf for 24 hours produced with considerable regularity 6 c.c. of fluid; in a shaded room and with a water-saturated atmosphere. This observation was repeated and verified a number of times, each time with adult froghoppers one day old in respect of emergence and in good health. One may therefore say on an average that a single froghopper will extract and void 1 c.c. of fluid in 24 hours. Froghoppers evince greater hunger if kept dry and die quickly if not given food, but it has not been possible as yet to observe a definite increase in amount of food taken in a dry atmosphere.

MacLeod (17) states that a drop of liquid is produced every 12 to 13 seconds and that an insect under observation excreted half a drachm (= 1·776 c.c.) of clear liquid in an hour. I have seen fluid excreted at greater rates than those which I have given above but I can quote no instance of such quantity as that given by MacLeod. Williams (29) refers to MacLeod and adds that with 50 froghoppers per stool, each sucking for eight hours during the night, about $1\frac{1}{4}$ pints of sap would be removed. I must confess that I hardly think that such a great drain often occurs in nature and I should expect that wilting and death of the cane would speedily follow under such conditions, without any intermediate symptoms such as are generally witnessed. No doubt, however, there is a considerable drain of fluid from the cane plant and this is an important fact to be borne in mind.

The fluid voided by the adult froghopper while feeding is an almost colourless and clear liquid. In quantity however it is seen to be faintly tinged brownish and to be slightly opalescent. It has a faint but disagreeable odour somewhat suggesting decaying vegetable matter or urine, but the odour seems most nearly to resemble that of certain amines or phosphorous compounds. The reaction of the fluid is slightly alkaline, *p*H 7·6 approx. It contains traces of various salts including phosphates, but apparently no reducing or other sugars.

The amount of fluid excreted by a froghopper gives one a reliable indication of the amount of fluid extracted from the plant[1], but it does not help one to determine whence this fluid has been derived, since all substances of nutritive value to the insect have presumably been ab-

[1] The amount of water vapour given off by the insect and plant in transpiration was shown to be negligible in the saturated atmosphere which obtained in these experiments.

sorbed from it before excretion. It might, however, be interesting to determine the amount of phosphorus in the excremental fluid quantitatively.

From the fact that saliva is frequently forced into the leaf during feeding it is obvious that its action is more than a purely lubricatory one. This would be expected from the results of previous workers on the Homoptera. Recent among these may be mentioned Davidson (6) and Horsfall (11) from whose papers earlier references may be obtained. Davidson, for instance, has shown that the saliva of aphids is diastatic in action, converting starch into soluble sugars. Petri (21), Zweigelt (30) and Staniland (24) record similar results. The saliva has also been shown to penetrate cell walls and even lignified xylem vessels. Collapse of the cells and plasmolysis of the cell contents often occurs. Frequently a stylet sheath is formed which Petri, Davidson and Horsfall suggest as being due to a reaction with the saliva.

The saliva of the froghopper shows very similar properties. It has a distinctly diastatic action upon starch and it contains, in addition, oxidases. Upon the cells and their protoplasmic contents it has marked effect, as will be seen. Apparently it has no power of inverting disaccharides. In reaction it is slightly acid, *p*H 6·0 to 6·2; Kershaw (14) states that it is neutral or faintly alkaline. Further and more detailed description of the salivary apparatus must appear in another paper of this series.

THE SUGAR-CANE.

The sugar-cane (*Saccharum officinarum* L.) is a large grass (Ord. Gramineae) which is generally propagated from sections of the stem. Raising from seed is not done in the course of ordinary cultivation. Underground rhizomes are formed from the original "plant" and these together with a mat of roots constitute the stool from which several aerial shoots arise. The aerial shoots or canes vary in length but they rarely grow to 20 feet. Leaves, which are long and narrow, arise at the nodes, but they have bases which completely ensheath the stem for some distance and therefore their points of origin may not be conspicuous. An eye, a potential branch, develops in each leaf axil and these axillary buds may be seen on the stripped (trashed) cane alternating, one at each node. As the cane grows the older leaves die and generally fall from the cane. Such stripped leaves are known as "trash" in Trinidad and elsewhere. In some varieties, as Uba, the leaves do not fall readily and the leaf bases remain clasping the stem. The canes are

commonly trashed when weeding and cleaning up of the fields is done and the trash is piled in heaps (boucans) between the rows of cane stools. In Trinidad the cane crop is cut, flush with the ground, during the dry season (January to May approx.). It then sprouts again (ratoons) from the stools and is once more cut after a year's growth. Plantings of stem sections are generally made during the wet season (June to December approx.). Plants arising from these sets are commonly allowed to grow for about 18 months before cutting. They are known as plant canes. After cutting, the next crop of canes from the stool is referred to as a first ratoon. Second ratoons and more may be taken but the practice of continual ratooning is not recommended and "plants" are best reinserted after the second or third ratoons on most soils.

The Leaf.

The leaves of the cane, which are alternate and two-ranked, consist each of an ensheathing base about 12 inches long which arises at a node, and an elongate leaf blade, from 3 to 4 feet long and 2 or 3 inches wide. The leaves, as also the stem, vary somewhat in colour and appearance according to variety and environment. They also vary in other details such as pilosity and in the relative abundance of siliceous serrations. All these differences may have some slight effect upon the preference of the froghopper, but this has not been well proved. They are probably far more important, indirectly in respect to froghopper, as affecting the degree of xerophily of the cane.

Internal Structure of Leaf.

There is a central midrib to the leaf which projects from the under surface. Above, it is devoid of chlorophyll and lies as a slight channel a little below the general surface of the leaf. In the lamina of the leaf run numerous parallel veins, of varying size, with cross-veins at intervals. Taking for example a mature leaf of B.A. 6032, longitudinal veins averaged 9 per mm. of width at the base of the leaf, 9 per mm. at the middle and 10 per mm. at the apex. They become more closely placed and of less diameter distally. Cross-veins averaged 9·5 per 10 mm. of length at the base, 8·4 per 10 mm. in the middle, and 6 per 10 mm. apically. Expressing this in terms of leaf area we have 855 cross-veins per sq. cm. at the base, 756 per sq. cm. in the middle, and 600 per sq. cm. at the apex; *i.e.* the number of cross-veins decreases apically. There is nothing remarkable in this but it may have some bearing upon water

conduction and certainly evidence points to the apex of the leaf being more responsive to froghopper punctures than the base.

In section of a cane leaf (Figs. 1 and 2) it is seen that the vascular strands are composed of xylem (*xy*) and phloem (*ph*) elements around which is a more or less distinct sheath of sclerenchyma fibres (*scl*). A more dense, characteristic mass of sclerenchyma fibres is interposed between the vascular bundle above and below and the epidermis (*ep*). Surrounding each vascular bundle is a border parenchyma sheath (*bp*) containing special chloroplasts which are grouped centrifugally. Again, applied to the border parenchyma sheath is one of chlorenchyma (*chl*)

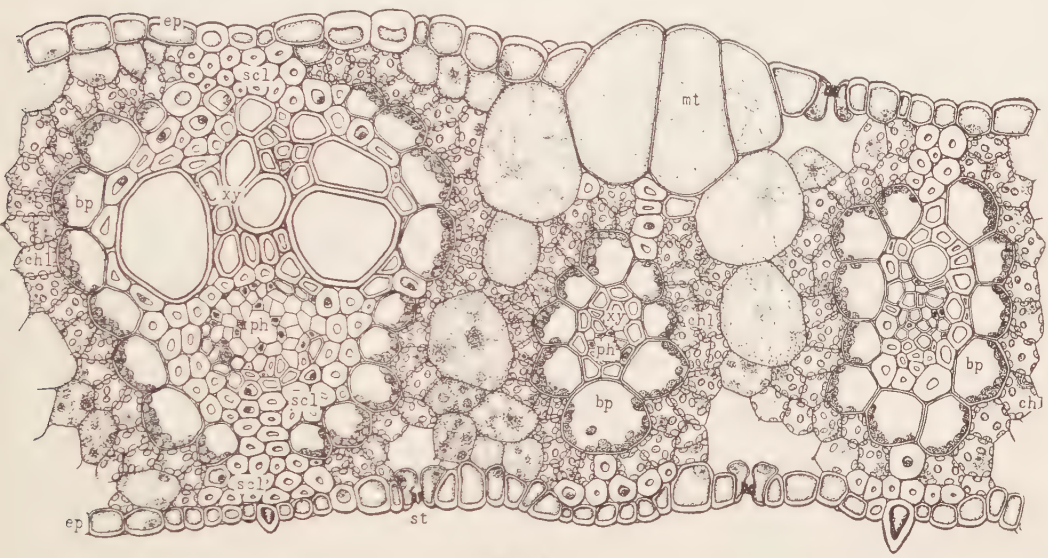

Fig. 1.

in which the chloroplasts are slightly smaller than those of the border parenchyma. The walls of the border parenchyma cells are a little thickened; those of the chlorenchyma cells are thin, showing intercellular air spaces in longitudinal section. The epidermis (*ep*) is more or less thickened and silicified. Stomata (*st*) are present on both upper and lower surfaces. Elongate groups of motor cells (*mt*) are to be seen. On the undersides of the veins are often silicified, tooth-like, epidermal cells, running like the teeth of a saw longitudinally.

As will be shown later, the tissues particularly of note are those associated with the vascular bundles, including the border parenchyma and chlorenchyma. The border parenchyma sheath constantly accom-

panies all veins and cross-veins. The slight lignification of its walls is proportional to the size and lignification of the vein which it accompanies. Thus around large veins the border parenchyma cells are more thickened than around small veins. Similarly with age the vein becomes further lignified as also does the border parenchyma sheath. It should be noted that the tissues are lignified, not suberised. They do not stain with Scarlet R, Sudan III, or chlorophyll solution. The cuticle of the leaf alone reacts to these dyes. The internal and transverse walls of the border parenchyma cells are pitted and probably allow passage of fluid. I cannot with certainty detect pits in the external walls which are contiguous with the chlorenchyma, nevertheless they may often be present here also.

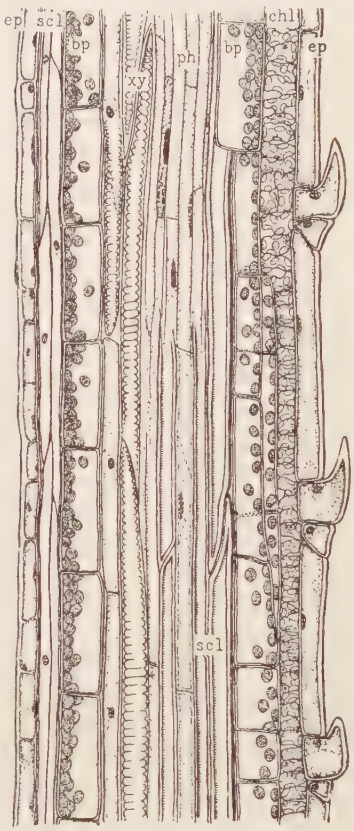

Fig. 2.

The border parenchyma of monocotyledons has, by various writers been compared with the endodermis or pericycle of dicotyledons and homology has been suggested. I do not wish to enter into any controversy upon such a point but the structure of the border parenchyma sheath and its behaviour when attacked by froghopper suggest some similarity in function with the endodermis of dicotyledons. Priestley and his co-workers have done much to clear up the physiology of the endodermis in dicotyledons. We need similar research upon the border parenchyma of the Gramineae.

Treatment of sections of cane leaves with iodine shows that the starch formed by photosynthesis first appears in the chlorenchyma, but about three hours after sunrise the plastids of the border parenchyma commence to accumulate starch. An hour later the border parenchyma plastids stain with iodine as darkly as the chlorenchyma plastids and from that time onwards the former plastids stain more intensely than the latter. This leads one to infer that the carbohydrate synthesised in the chlorenchyma is passed on continuously to the border parenchyma plastids which act as temporary reservoirs during passage to the vascular

strands. It is unlikely from the position and structure of the border parenchyma plastids that they can be more efficient photosynthesising organs than the chlorenchyma plastids. In the dark the starch is rapidly hydrolysed and doubtless passes into the vascular strands through the pits or perforations already noted.

The contents of the border parenchyma plastids have been stated to be starch; it is more likely, however, that the carbohydrate here is not quite in this advanced stage of condensation. The plastids really stain dark brown to black with iodine and not blue at all. Sections of leaves placed in distilled water rapidly lose their iodine-staining components. This loss may be due partly to hydrolysis from enzyme action, or the substance staining brown may be water soluble, *i.e.* amylodextrin or erythrodextrin. Hydrolysis probably occurs in addition to simple solution. Leaves extracted in bulk for starch, after first killing with hot alcohol, yielded dextrins and no starch.

Although the structure and reactions of the cell walls do not definitely indicate the radial and tangential walls of the border parenchyma cells to be more impervious, except possibly in the scarcity of pits, yet passage of fluid is obviously easy longitudinally through transverse walls and much less through radial or tangential walls when under the influence of froghopper saliva. This will be referred to again.

Reactions of the Vascular Tissues.

Fresh leaf sections in distilled water were treated with the usual series of indicator dyes. The phloem, cell contents of the border parenchyma and the chlorenchyma appeared to have a *p*H value between 5·6 and 6·0 (using methyl red, diethyl red, and brom cresol purple). Various interstitial parenchyma was slightly more acid, but varied in the region of *p*H 5·2. The walls of the phloem cells were often slightly more acid than the phloem contents. Xylem and sclerenchyma showed a decidedly acid reaction, red with methyl red and pale yellowish with brom phenol blue and thymol blue. Their *p*H seems to lie between 3·0 and 3·6.

These effects with dyes are generally accepted as reliable indices of the hydrogen ion concentration of cells and cell contents, but there are many possible complications resulting from adsorption and chemical change. The results are offered for want of better methods[1]. It is to be

[1] Fresh leaf sections were treated with $FeCl_3$ for five minutes, washed with water and potassium ferrocyanide solution added. Phloem and chlorenchyma alone stained blue and should from this be considered acidic. On adding HCl, of course, all tissues stained blue. These would be expected to be more basic. These results are difficult to reconcile with the effects of indicator dyes.

expected that the enzymes contained in froghopper saliva, as other enzymes, act most efficiently within a certain range of hydrogen ion concentration, probably near the isoelectric point of the saliva proteins(23); at least here is a possibility which cannot be ignored.

In addition to what has been said of the localised carbohydrates in the leaf, other constituents which should be noted are the oxidases and tannins. With guaiacum and other oxidase reagents such as α-naphthol and benzidine, oxidases can easily be demonstrated in fresh sections of leaves and other tissues. It often happens that with tincture of guaiacum alone certain cells of the intervascular regions are tinted slightly blue, but the entire leaf section may not be coloured until the addition of hydrogen peroxide. It may therefore be more correct to refer to the majority of the enzymes present as peroxidases although the slight occasional blueing at first suggests that possibly the guaiacum reaction is merely being inhibited by such as tannins or sugars also present in the leaf tissue.

Catechol tannins can be demonstrated in fresh leaves and many other parts of the plant. In some varieties of cane the tannin content is high. The tannins are soluble in water. Their solution gives a greenish-black colour with ferric chloride and a precipitate with bromine water. Fresh sections of leaf treated with ammonium molybdate in ammonium chloride give a deep yellow precipitate almost alone in the motor cells, but the contents of these cells are peculiar and give other special reactions. Nearly all leaf tissues react with $FeCl_3$.

Effect upon the Leaf of Froghopper Sucking.

The adult froghopper in feeding punctures the leaf near a vascular bundle, as has been said already. The stylets may break into the vascular strand or they may only injure the surrounding tissues. The puncture made is not a neat one (Fig. 3). It frequently passes almost through to the opposite side of the leaf, completely through in some soft grasses, and is often very destructive mechanically to neighbouring tissues. Saliva is pumped into the wound and sucking commences, roughly, it may be assumed, when well in action, at the rate of voidance of liquid excrement. The length of time of feeding at one puncture varies, and after effects on the leaf also vary, but only to a certain extent correspondingly.

Examining the leaf at once and while fresh under the microscope, the puncture is visible and around it, if feeding has been at all prolonged, there is a distinct bleaching of the chlorophyll, more longitudinally than

laterally. A small amount of dried saliva may be visible around the aperture. The vein or veins themselves near the puncture, extending for a variable distance longitudinally, often appear paler or sometimes watery by transmitted light. At other times the vessels seem to contain air drawn in from the point of puncture; probably on release of the tension produced by the insect's sucking. Testing the spot with a cobalt chloride tripartite strip the rate of transpiration is often found to be more than doubled. This may be, and very likely is, due purely to an oozing from the seat of puncture and it is not necessarily connected with increased stomatal aperture or with increased metabolic rate.

Fresh sections cut through the spot have, on very few occasions, shown a slight change in *p*H, in the direction of increased acidity, viz. the contents of the border parenchyma cells appear slightly more orange-pink to methyl red—a never very reliable indicator. With iodine there is no darkening in the region of the puncture when a distinctly positive reaction is obtained in other parts of the leaf. It is probable that the carbohydrates of the cane leaf are always easily extracted as they appear to be in the form of dextrins which are water soluble.

In a few cases where the sucking of the froghopper had continued for an hour or more, certain cells showed a slight pink or brown coloration, due probably to oxidation of catechol tannins, as will be mentioned again later.

Leaves were fixed in a variety of fluids at varying times after the cessation of feeding and with known duration of feeding. The most striking effect noticeable even after only five or ten minutes' sucking is upon the contents of the border parenchyma cells. Other damage, apart from the purely mechanical rupture and the formation of a stylet sheath is of less apparent consequence at first. When the excretion of faecal water has been rapid the vascular strands are invariably found to have been broken into and their contents presumably drawn upon.

The puncture made by the stylets is fairly straight, broad and somewhat conical (Fig. 3)[1]. Its walls are lined with a peculiar material which arises probably as the result of interaction between the insect's saliva and certain plant substances. This lining has been referred to as the stylet sheath in connection with aphids, and various workers, Petri (21), Wells (27), Davidson (6), and Horsfall (11) have demonstrated the presence

[1] The illustration, Fig. 3, shows a section of *Paspalum* leaf. This is soft and thin, unlike a cane leaf. Penetration of the froghopper's stylets is slight and easy, and the puncture is distinctly conical. In a cane leaf the puncture is much straighter and longer, and it is less markedly widened at its orifice.

of pectic materials in this sheath. Calcium pectate especially has been mentioned, besides proteins and tannins. The present writer has obtained a staining with ruthenium red and also with methylene blue,

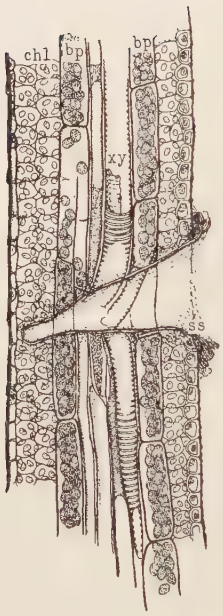

Fig. 3.

Fig. 4.

after treatment with HCl, but only occasionally. Other general reactions seem to agree with the results of the above-mentioned writers, but further investigation is intended upon this. Tannins have always been

detected in the stylet sheath produced by the froghopper on cane leaves.

With safranin O, counterstained by picro aniline blue or water blue, the stylet sheath and certain tissues immediately connected with the puncture show rather intense safranin staining as a rule. The cell contents of normal, unaffected, border parenchyma cells stain blue, although their walls, especially if thickened, are stained red by the safranin. Affected border parenchyma cells are stained wholly red by the safranin and show in marked contrast to the unaffected cells and chlorenchyma. The safranin staining runs strictly longitudinally in the border parenchyma where salivary action has been of short duration and it passes from one vascular sheath to the next along the cross-vein sheaths. The effect, in longitudinal section of the leaf, is most peculiar. There may be a long line of brick-shaped border parenchyma cells stained red and a parallel, contiguous line, in the same section, stained blue and unaffected by the saliva. In section of the next parallel vein, affected border parenchyma cells run longitudinally from the points where cross-veins connect (Fig. 4), so that a few veins away from the puncture laterally only isolated border parenchyma cells here and there are seen stained by safranin. If one were to judge by stained sections alone, one would consider the froghopper's saliva to be specific for the contents of the border parenchyma cells and this is the more interesting in view of the probable great physiological importance of the border parenchyma sheath. The border parenchyma certainly accumulates carbohydrate materials temporarily, as is evident from its deep staining with iodine in the daytime, and these carbohydrates probably pass from the border parenchyma into the vascular bundle at night. Again, since the border parenchyma sheath is interposed between the vascular bundle and the chlorenchyma cells one may well postulate that water, salts, etc., from the vascular strands have to pass through the border parenchyma sheath to the chlorenchyma. The chlorenchyma around a froghopper puncture allows of slight spreading of the insect's saliva, probably due to the numerous air spaces in this tissue, but in the border parenchyma the saliva passes rapidly in a longitudinal direction. If the transverse walls of the border parenchyma were absent one could hardly expect a more rapid longitudinal spread. Lateral extension is however slow in the border parenchyma and is usually associated with prolonged salivary action.

The remarkable effect of the saliva upon the contents of the border parenchyma may very likely be due to the fact that the enzymes of the

froghopper's saliva exert their greatest activity at the H-ion concentration of these cells. If this be true then it is to be remembered that the phloem has a *p*H value nearly in the same region, while the chlorenchyma is slightly more alkaline and the xylem more acid. The air spaces in the chlorenchyma will tend to hinder extension of saliva in that tissue, as has been suggested already, but the phloem and border parenchyma certainly suffer severely. Xylem and sclerenchyma do not show damage so readily.

Sherman (23) has shown that malt diastase exhibits maximum activity at its isoelectric point, which is between *p*H 4·3 and 4·5. Now the enzymes of the froghopper are also likely to be proteins and they may have isoelectric points in approximately the same region. It is quite likely that the xylem and sclerenchyma on the acid side and the chlorenchyma and other tissues on the more neutral side are not so markedly affected at first because of their *p*H being outside the optimum activity zone. This is put forward as a possibility, but further investigation along these lines has yet to be made.

In the case of short duration (5–15 mins.) sucking, then, one may say that the main apparent effect is upon the plastids of the border parenchyma cells. The effect upon the vascular tissues is less noticeable and that upon the chlorenchyma very local. There may be slight plasmolysis.

With prolonged sucking the effect is more widespread. Chlorenchyma and vascular tissues become more involved. The blanching of the former has been already mentioned and this is usually followed by death of the cells, which no longer show photosynthetic activity. It is a mass effect which is to be expected and it is of less interest in explaining the action of the froghopper's saliva *per se*, since post-mortem changes with consequent autolytic and other effects occur. These mass effects must and will of course be considered as the feeding punctures from which spreads the typical and serious leaf damage are invariably the result of prolonged sucking.

From the slight damage such as has been first described a leaf may or may not recover, according to its condition. A normal healthy leaf, under good conditions, usually recovers completely. After a few days the green colour of the leaf, if it had been slightly bleached locally, is restored, probably by regeneration of plastids, and sections of the leaf indicate that it is again in normal functioning state. Under less favourable conditions, such as will be indicated later, especially when coupled with prolonged sucking of the insect, the following external changes may be visible.

Paling of the green parts of the leaf extends, mainly in a longitudinal direction, on either side of the puncture, but often more towards the apex of the leaf than towards the base. Extension of the injury is often accompanied by the appearance of a red pigment which seems to be the result of oxidation of catechol tannins already mentioned as being present in the leaf. The appearance of red pigment varies with the variety of cane and with the conditions under which the cane is growing. Thus in B. 156, on certain heavy soils, the red pigment may hardly be visible at all. The injury spreads as a yellow, somewhat ill-defined streak for a time and then becomes dead and brown in the centre. The yellow portions continue to advance followed up by the brown dead central area. Usually, however, the red pigment is also seen in B. 156 although maybe to a less extent than in other varieties. Uba, and other varieties, under good conditions, and often under bad, may show little yellowing. Within a day or two after puncture a red, finely stippled streak appears. This elongates and the centre later becomes brown and dead. Peripheral yellowing of the streak may later be visible.

The case of a fairly healthy leaf B.A. 6032 may be taken as an example. Two days after puncture, with two hours of sucking by a froghopper, there was a pale streak, very narrow and indistinct, 0·5 cm. long, showing some minute red stippling. In three days the length was 0·8 cm., almost entirely red. Next day the red streak reached 3 cm., and it continued as below:

5th day	...	...	5·0 cm. × 0·15 cm.
7th day	...	...	6·8 cm. × 0·25 cm.
8th day	...	...	9·5 cm. × 0·25 cm.
9th day	...	...	11·0 cm. × 0·30 cm.

On or about the 9th day the centre of the red streak appeared brown and dead. Yellowing was visible in some places at the periphery of the red area. Elongation continued. On the 11th day the streak measured 13·5 cm. × 0·45 cm., and on the 14th day it was 18 cm. × 0·70 cm., dead and brown with little or no red pigment. Following upon a yellow margin, 1 to 1·5 mm. wide, the dead leaf area continued to extend.

The case cited is only one of many. There is much variation with variety of cane and with environmental conditions. A detailed study of these varying symptoms seems likely, from observations already made, to afford valuable clues to the nature and mechanism of recovery from or resistance to attack, but such study is beset with many difficulties, especially in the standardisation of conditions, and results so far ob-

tained, while interesting, cannot be regarded as necessarily significant. Much and prolonged yellowing of the leaf is frequently followed by more extensive subsequent death of the tissues. Production of red pigment and reduction of yellowing is commonly a more favourable reaction, indicating resisting vigour.

While the external symptoms just mentioned are developing, certain important internal changes responsible for them have occurred. The immediate effect of the froghopper's saliva upon the border parenchyma has already been described, but there is less immediate effect apparent upon other tissues unless feeding has been much prolonged, in which event blanching of the chlorenchyma and sometimes faint local reddening, due to oxidase action, may be seen.

After a day or two the cells of the phloem are seen to have deposits within them, probably of pentosans, which give a tannin reaction: tannins are, however, present in nearly all tissues. These deposits are often associated with red pigment. The chlorenchyma cells around such affected vascular bundles are apparently dead or dying. Water supply to them is obviously checked, since leaves, after injury by froghopper, when placed in eosin solution show conduction of the dye to be prevented or greatly reduced wherever injury has spread, whereas normal and uninjured veins conduct the solution freely and rapidly. This effect extends in a longitudinal band apically. A transverse section through an early red streak (three days or more old) shows the phloem almost completely blocked with reddish material and the walls of xylem and sclerenchyma in the same bundles stained red, but with lumina not usually at all blocked. Death and disorganisation of the surrounding tissues is obviously in progress.

The red pigment found in damaged leaves is also formed, with varying rapidity, when any aerial part of the cane plant is cut and exposed to the air, and, as has been said, it is probably the result of oxidase activity upon catechol tannins. The red pigment is slowly dissolved out in cold water, more rapidly on heating. Upon addition of ammonia there is a colour change to yellow. Reactions of a catechol tannin are given by the red pigment and it seems probable that the pigment is an oxidation product of such tannins. Further, and much more detailed examination is necessary before a conclusion can be reached. Wolzogen Kühr(16) states that the red decomposition product of cane juice is purpurin, 1 : 2 : 4-trihydroxyanthraquinone, or a very nearly allied substance. Petrie(22) has investigated the red pigment produced in *Eucalyptus* consequent upon injury due to *Eriophyes*

eucalypti and considers it to be derived by decomposition from a catechol tannin. Several reactions are given by him, but the cane pigment appears to differ from that which Petrie had under examination.

The spread of injury from a froghopper puncture would then seem to be explained somewhat as follows. Firstly, besides the direct injury and drain upon the contents of the vascular tissues, there is a marked injurious effect upon the border parenchyma, with removal of carbohydrates, etc., stored temporarily therein. The injury to border parenchyma is extensive longitudinally. The immediate effect upon chlorenchyma is more localised. The injury produced by the saliva is effected by several ferments of which diastatic and oxidising enzymes have been demonstrated. Probably others exist. The metabolic equilibrium of the plant is upset. The rate of respiration probably increases locally with oxidation. Supernormal oxidation with the formation of red pigment occurs from the action of plant oxidases possibly aided by the oxidase in the insect's saliva. Transpiration has, on a few occasions, been shown to increase in the injured region, but not always.

Loss of sap with its contained food materials from the vascular tissues can be made good by translocation from elsewhere, but local translocation has been interfered with and probably arrested by the injury to the border parenchyma sheath. This results in reduction of water supply, with solutes, to the chlorenchyma and other neighbouring tissues and unless loss of water, etc., is reduced by decreased transpiration a local shortage in the tissues is likely to result. There is, however, certainly no decrease in transpiration rate. Interference with translocation is further intensified when the phloem becomes blocked. Water deficiency prevents recovery of the protoplasts in all affected cells and culminates in a drying up of the tissues. This slow desiccation extends unless the local rate of water supply can be increased or unless water loss by transpiration can be reduced. Assuming continued effect of the enzymes introduced by the froghopper, the metabolic equilibrium of the plant will continue to be upset unless a sufficiently powerful counter-reaction is forthcoming. This counter-reaction is likely to be impeded by difficulties of conduction.

As regards the possibility that the froghopper may be the vector of disease organisms; this was considered early. Juice from parts of leaves showing froghopper injury was inoculated into healthy leaves; small pieces of dying tissue were inserted into healthy leaf tissue, but all without success. Froghoppers bred from the egg upon healthy canes were still as adults capable of producing typical leaf injury. These

experiments suggest that disease transmission is unlikely although they do not disprove the possibility of such.

Conditions Influencing Extension of Leaf Injury.

As one of my earliest experiments a healthy cane, growing in a large concrete pot, was placed in a wire gauze cage, shaded above, and between two and three hundred adult froghoppers were released in the cage. They gradually died off in a few days and I was surprised to find that only one small typical froghopper streak was finally produced upon the cane. Lesser injury was discernible with difficulty.

Urich (26), p. 10, says: "By way of testing the action of feeding of adults on cane leaves, a young and vigorous cane plant whose roots were free of nymphs was isolated and during a week 100 adults were introduced every day into the cage. A month after the leaves did not show any signs of blight, but they bore a few yellow spots, not enough to disfigure the plant."

On the other hand, Williams (29) was successful in producing typical signs of blight upon a healthy cane by caging froghoppers over it.

In an endeavour to determine the reasons for the above contradictions a number of experiments were made with the result that further inexplicable discrepancies were encountered. Results were sometimes positive, sometimes negative. Most even results were obtained with small plants from single eye sections bearing five or six leaves and few roots, but the damage was often not quite of the same type as field damage. Usually the injurious effects were more rapid in their spread. The leaf yellowed and showed dead tissues often in a week after puncture. Frequently the whole leaf rolled and died rapidly back from the apex. It was decided that these symptoms were really due to similar causes to those producing the more typical damage in older canes with larger food reserves and better root systems and therefore, as older canes were uncertain and difficult to manipulate, experiments were carried on with young cane plants such as have been described.

Six small B.S.F. 12.27 plants, growing in pots in local soil, which has the consistency of a clay-loam, were selected. Of these three were kept with reduced water supply for two weeks to such extent that the leaves tended to roll, especially at midday. This shortage of water was kept as near constant as possible without any auto-irrigation device. The other three control canes were watered lightly and regularly, so that the soil was moist but not waterlogged. Six froghoppers were placed in gauze-topped cylinders over each of all six canes for 12 hours

overnight. The cylinders with froghoppers were then removed and conditions of growth maintained as before. The canes short of water showed signs of injury after two or three days; they paled, and later the tips and margins of especially the older leaves withered and died. On the other hand much less signs of injury appeared on the well-watered plants. They seemed little worse after their exposure.

The experiment was repeated with B. 156, and with similar results, but it was thought that possibly the froghoppers under dryer conditions sucked more than those in the fairly saturated atmosphere over damp soil. (It is easily shown that the adult froghoppers become hungry more rapidly in a dry than in a water-saturated atmosphere.) A single froghopper was therefore enclosed upon one leaf and was allowed to feed for 10, 20, 30 or 60 minutes, when it was removed. Absolutely consistent results were not obtained, but on an average the leaves of well-watered plants showed little appreciable injury after up to 20 or 30 minutes' sucking, although a slight local yellowing occurred in one or two cases after 30 minutes' sucking. On the other hand 20 or 30 minutes' sucking often caused later spread of injury, not serious, upon leaves of canes lacking sufficient water. It was found, however, that the tips of leaves were apparently more susceptible to injury than the bases, also the older the leaf, after prime, the more susceptible to injury it became. With additional experiments it was concluded that results obtained by allowing the insect to suck for a given time were not reliable. Too many exceptions and irregularities occurred. The first method of experimentation, using six or twelve insects overnight, was therefore again resorted to.

Plants growing in washed quartz sand only, showed less susceptibility than those grown in a heavy clay soil from a blighted field. It should be mentioned that both series in this experiment had pieces of parent cane, with its food reserves, still attached. Both had ample water.

Reduction of roots, by cutting several of them through near the base of the plant, had the effect of increasing susceptibility.

Exposure of previously attacked plants to full sunlight increased extension of injury, while shade from direct sunlight had a beneficial effect and aided recovery from the effects of sucking.

Plants in waterlogged heavy soils did not appear to be more susceptible than control plants in the same soil but with less water.

In all these experiments attention of course was paid, as far as possible, to the standardisation of conditions other than the variables under consideration.

All the above results are not considered by the writer as proving that water shortage is a predisposing cause of increased susceptibility to froghopper injury, but they at least point in that direction. Many more experiments, under better controlled conditions, are necessary before any proof can be considered to have been established. Such experiments are in the course of arrangement and among other plans it is hoped to set up a series of canes under controlled atmospheric conditions with auto-irrigators to control soil moisture, etc. These experiments necessitate much careful preparation and manipulation and opportunity has not yet been forthcoming to carry them through satisfactorily. Preliminary experiments reveal several objections to the type of auto-irrigator commonly employed for the present work, and these objections have yet to be obviated. For instance, many roots of the plant grow round the porous pot water source, thus obtaining water direct from it instead of through the medium of the soil. This reduces any toxicity effect of the soil.

Increased susceptibility of cane to other diseases, besides insects' sucking, has been observed by the writer in Trinidad. Thus *Coniothyrium* sp.n.[1], which causes a leaf injury often very similar to that of froghopper, only makes its presence conspicuous towards the end of the dry season.

Again, a disease of unknown origin[2], which may be described as a localised leaf rash, does not appear until the end of the dry season, and even then only upon certain varieties of cane. The writer first noticed it upon B. 156 upon which it appears as a rash of small red spots running on either side of the midrib of the leaf. The individual spots are at first only about a millimetre in length, but later may be 1 mm. × 2 mm., although varying considerably. The spots are closely placed together and the rash extends as a rule for a length of from 2 cm. to 10 or 15 cm. by from 5 mm. to 25 mm. in breadth. The spots may become slightly confluent, but the disease does not appear to exert any seriously deleterious influence. The present interest of the disease lies in the fact that it can be inoculated into other B. 156 plants, but not if they are well-watered and healthy. Sometimes the plant will show a slight stippling. The disease is never visible in the wet season. It can also be inoculated into other varieties of cane with slightly varying results, but varietal

[1] About to be described by Dr J. R. Weir, of the Bureau of Plant Industry, U.S. Dept. of Agriculture.

[2] Spores similar to those of *Leptosphaeria* have been found in the diseased tissue, but they have not been shown to be related to the cause and are quite possibly secondary.

resistance is shown and in all cases the canes must be deficient in water to show effect. In one of the College seedlings the midrib shows a red coloration as well as the rash on either side. In another the principal effect is a red coloration of the midrib with little or no rash. These varietal responses are most important and should be studied further. Various other reasons for disease resistance in plants have been assigned by other investigators. Some of these will be considered later.

It is very fortunate that the froghopper requires damp conditions for breeding and that it does not appear as a rule in any numbers during the dry season. Were this otherwise it is certain that the damage done would be very great in time of severe drought.

It has been said that old leaves appear to be slightly more susceptible to injury than those not past their prime. As a leaf increases in age lignification of the vascular tissues increases in proportion. One might expect that this would prove a greater obstacle to the froghopper in piercing the tissue. As to preference shown by the insect for old or young leaves I can offer no evidence, but it seems clear that changes associated with increased lignification of the leaf render the latter less able to recover from the effects of the insect's sucking. This may be purely an effect of senescence and generally lowered vitality. It may also be that the passage of water in the vascular tissues is impeded by lignification.

Also, it was noted that the apical portions of the leaf died more rapidly than the basal portions after froghopper injury. It seems obvious that the basal portions of the leaf are better supplied with water than the apical ones, and I would also restate the fact that cross-veins are less frequent apically than basally.

The Root System.

The root system of the cane varies, as might be expected, with the variety of cane and with soil conditions. Generally speaking there is a dense mat of roots which are fairly near the surface and which usually vary in length between 5 and 12 inches. These constitute the principal roots of the stool. A few roots, however, penetrate much more deeply and travel directly downwards. Such roots may reach a length of several feet. Sometimes there may be only two or three such roots, at other times a dozen or two.

A number of cane varieties were grown for a year under fairly good soil conditions in the College grounds. At the end of this period the soil was carefully washed away with water and the roots examined.

Individual stools varied to a certain degree and it would be difficult to pass an opinion on the six or seven stools of each variety examined. Many had really good root systems and this remark would apply to most of the generally favoured varieties. Uba was particularly good with stool roots averaging 10 to 12 inches long and about a dozen roots of 2 to 3 feet or more in length. The observations were unfortunately complicated by the presence of *Leucotermes tenuis* which had badly attacked the bases of the stems of several varieties, especially Badilla.

A few stools were examined, as far as was possible in the very heavy, low-lying clay soils which suffer so heavily under froghopper and in these the roots appeared to be very superficial. In nearly every case D. 116, B. 156 and B. 347 had very slight hold of the ground and the root system was extremely poor. Many roots were dead. On such very heavy clays Uba certainly grows better than any other variety.

In all these heavy soils a large percentage of dead roots was noticed, but as the majority of stools examined were first and second ratoons it would not be advisable to stress this point until further and wider observations have been made. Root fungi were also often noticed.

Nowell (20), paragraph 11, observed that dead roots were found in blighted stools, but he adds that the same applied to many uninfested ratoon fields in poor and compacted soils. It has often been noted that roots are killed by prolonged lack of aeration and also by various toxic products in the soil. I am inclined to believe that such uninfected fields as Nowell mentions, with poor root systems, would have suffered badly had they been attacked.

In view of what has been stated as regards the increase in susceptibility the nearer the cane approaches its wilting point, it is unnecessary to stress the vital importance of the roots. In a dry soil the roots obviously have greater difficulty in obtaining water than in a moister one, but it is also well known that in heavy, waterlogged soils, with reduced aeration, a condition of physiological drought obtains and plants behave as though actually deprived of water. It has been observed by Nowell, Williams and others that froghopper is likely to be serious on waterlogged, heavy clays and this is bound to strike any investigator of the froghopper problem. The soil question will receive further consideration later.

It is noteworthy that with canes in fields subject to froghopper or especially in fields after froghopper attack, as observed by Williams (29), adventitious roots are frequently sent out from the nodes. This is a natural response of a cane with soil root system inadequate to meet

the demands made by the aerial portions of the stem, or else it may indicate faulty conduction in the basal portions of the stem. It is probably associated with changes in the osmotic pressure of the cell sap and is consequent upon the increased acidity and enzyme action which results from serious froghopper injury. Whatever may be the cause, the upper portions of the plant are clearly in a state of deficiency.

Added to a poor root system is often the effect of root disease. Root fungi more frequently attack weak canes than healthy ones, especially old and used up ratoons. Roots with root disease are reduced in efficiency and water shortage to the plant is a natural sequel. Root fungi and froghopper may therefore often be interdependent. Canes weakened by the fungi are more susceptible to froghopper and also canes weakened by froghopper, especially possibly by the nymphs, are more susceptible to root diseases.

Recovery after froghopper requires a good root system and plenty of available water. Laboratory experiments have shown that serious reduction in the number of roots is inimical to recovery and the same undoubtedly holds in the field where roots have been reduced and killed by root fungi. A shallow root system such as is formed in fields of badly aerated heavy soil, especially when low-lying and waterlogged, is very inefficient and in slight drought, when it can obtain no surface water, it is considerably handicapped by the absence of deeper roots. Also it should be remembered that the percentage of water non-available on a heavy clay soil is considerably higher than that of a better aerated soil.

The Stem.

The stem of the sugar-cane is cylindrical and is divided into nodes and internodes. Leaves arise alternately at the nodes and in each leaf axil is an eye or dormant bud. Also from the nodes adventitious roots develop. The internodes of the stem are smooth, with hard, variously coloured rind, often covered with wax. A cross-section of an internode shows that the vascular bundles, which run parallel to each other embedded in parenchymatous tissue, are more numerous, though smaller, at the periphery than in the centre of the stalk. The stem serves for conduction of fluids, with solutes, and for storage of sugars and other reserves.

Effect of Froghopper Blight upon the Stem.

When froghoppers have attacked a cane the leaves die prematurely, as has been described, and as a result photosynthesis receives a check.

The stem, deprived of photosynthetic products, grows more slowly and the internodes laid down while the cane is in an injured condition are shorter and narrower than those produced by normal growth. The internodes are also usually brittle and snap readily immediately above the nodes. Williams (29) showed that an exactly similar effect upon the stem was produced by cutting off the leaves of the cane and this experiment has been repeated and confirmed by myself. It is clear that injury to the stem is due to the check which reduced leaf metabolism gives to the plant by failure to continue the supply of elaborated food materials.

There are other changes which occur in the stem consequent upon death of the leaves. These are largely due to enzyme activity, the enzymes being resident mainly in the tops of the canes. By oxidation a red-brown discoloration is often produced, similar to that noticed in the leaves, but more striking is the change in reaction of the cane sap, with hydrolysis of sugars, etc. Normal cane sap varies slightly in reaction with age and variety of cane, with the soil upon which it has been grown, and also with the region of the cane from which the juice has been extracted. Care has to be exercised in taking *p*H readings not to expose the sap unduly to air and to avoid delay. Using Clark and Lubs' indicator dyes the reaction of a healthy cane sap is found to lie between *p*H 6·0 and 6·3, when grown in soil in the College vicinity. The young parts of the top of the cane often have a slightly greater acidity, *p*H 5·9 or so. Canes grown on heavy clay soils, often when less acid than the College soils, showed greater acidity.

With injury by froghopper and death of the leaves the *p*H of the cell sap falls gradually, starting at the top of the cane. In fairly good soil it rarely drops below *p*H 5·7, but on some heavy soils it may be as low as 5·4. This increase in acidity is most noticeable at the top of the cane where enzyme activity is greatest. Following upon increased acidity the top of the cane gradually dies and the terminal bud rots. Shoots may be sent out from eyes near the base of the stem. This final condition is characteristic. With continued post-mortem changes in the top of the cane stem the *p*H rises again and it may become less acid than a normal healthy cane, some time after death. Death of the terminal bud, when not itself directly injured by the froghopper, is undoubtedly due to interference with supplies from the stem and the formation of toxic products during the course of pathological changes in the stem. Shooting from the lower eyes is to be expected as a sequel to death of the growing point and alteration in the osmotic pressure of the cell sap.

Recovery of the cane, so far as the stem alone is concerned, is dependent upon an arresting of the pathological changes above described. A cane which is likely to recover does not show much increased acidity after the leaves have died, but it is questionable whether a cane with more highly buffered juice would be more resistant than one with a less highly buffered juice. Uba is noteworthy as being a very vigorous cane and one which is resistant to many diseases under difficult soil conditions. It stands up better than any other variety under froghopper attack. One may associate with this vigour the fact that the juice has an unusually high colloid content, only too well known to sugar manufacturers as increasing their difficulties in clarification. We are therefore forced to recognise the possibility that the more resistant a cane is to froghopper the less acceptable it may be to the manufacturer.

The importance of good conduction in the stem to resistance against froghopper attack was most strikingly illustrated by the single case described below. In a patch of farmer's canes at St Augustine on February 1st, 1925, one stool was noticed bearing four canes. Of these canes, one was slightly taller than the others, but it bore somewhat smaller leaves and was obviously in poorer condition. The remaining three canes of the stool were fairly vigorous. There was evidence of slight froghopper attack throughout the whole cane plot. The striking feature of the stool noted was that the slightly taller cane showed much froghopper damage on the leaves while the three smaller but more vigorous canes were quite insignificantly damaged. This was totally unexpected, a varying resistance within one plant, and the cane was therefore carefully examined. The roots of the stool were apparently quite healthy. Examination of the stem showed that the taller cane was badly bored by *Diatraea* larvae while the smaller canes were untouched. The explanation of this phenomenon would seem to be that conduction had been interfered with by borer to such an extent in the taller cane that the leaves were in a state of water shortage. The three healthier canes with good stems did not show such suceptibility to froghopper injury. It may also be added that the bored cane showed more acid sap than the unbored canes, as was to be expected.

Carmody (5) noted the coincidence of local attack by the large moth borer, *Castnia licus* and froghopper at Caroni in 1908. Nowell and Williams have mentioned other cases with *Diatraea saccharalis*, but they refer to the borer attack as a complication of the froghopper attack.

Effect of Soil Conditions.

The optimum soil conditions for sugar-cane are apparently unknown. We do not even know definitely the range of H-ion concentration which is most conducive to good development. With such ignorance it is obviously impossible to proceed very deeply into the effect of soil conditions upon froghopper attack.

It has been mentioned by Williams and others that attacks from froghopper are worse upon acid soils and evidence has been adduced in support of this opinion. The present writer was struck by the possibility, after a perusal of existing literature, and therefore made a preliminary survey of the reactions of Trinidad soils subject to froghopper attack. A Wherry double-wedge comparator was used in most of these investigations and was found to give sufficiently accurate results for the purpose. Its ease and relative rapidity of manipulation was a distinct advantage. From these preliminary studies it became evident that acidity alone had little to do with susceptibility to froghopper attack and that the *p*H recorded from most bad fields could not be considered as injuriously acid. Moreover when attention was turned to the good fields many of these were found to be equally acid or even more so than the bad ones.

In Williams' time and before, the method of testing soil reaction was by means of litmus paper and the selection of soil samples was not in accordance with the more careful modern methods. The sources of error with litmus are well known.

In the present cursory survey it was found that several heavy clay soils on lands attacked badly by froghopper were even alkaline in reaction and a *p*H of 8·0 was by no means rare for some reddish and some grey-black clays. Many of the very bad red clays were neutral. The acid soils were often not more acid than *p*H 6·5. Further north in the island, on lighter soils which are hardly subject to froghopper, the *p*H was found sometimes to be as low as 5·5. A *p*H of 6·5 was perhaps the average for the samples taken. Without going further it was obvious that acidity of the soil could not be correlated with froghopper blight and this line of research was therefore no longer purused. There is much work, however, to be done and a soil survey of Trinidad upon modern lines is badly needed. It is useless to attempt to draw conclusions from the casual examination of a few blighted fields when the conditions obtaining in good fields are unknown.

The opinion has been expressed by both Williams and Nowell that

much of the remedy for froghopper blight lies in the improvement of soil tilth. It is considered, and rightly, that canes grown upon soil in a good state of tilth will be healthier and more readily able to withstand the effects of froghopper attack than those grown upon soil in bad condition. The need for sound agricultural methods cannot be overemphasised in Trinidad, but improvement of the physical condition of many soils in the island will be a long and tedious process. Some of the most noteworthy froghopper soils are incredibly heavy clays and it is indeed remarkable that canes will grow at all upon them. Speaking of certain lands well known for their predisposition to blight G. W. Robinson, of University College, Bangor, says (in litt.) "The Cedar Hill black subsoil is the heaviest clay I have ever seen. I don't think we could show such heavy soils in cultivation in this country. If there are soils as heavy they are down to grass."

In the great majority of cases the most serious froghopper attacks occur upon heavy compacted soils in thoroughly bad physical condition. It is in no way surprising that canes growing upon such soils are frequently in a state of physiological drought. It is a well-recognised fact that plants growing upon waterlogged and oxygen deficient soils behave as though in a state of water deficiency. Interference with water absorption by the roots is one of the first effects of the removal of oxygen. Xerophytic adaptations are characteristic features of plants native to badly aerated soils. In the case of some of the soils noted in Trinidad it would seem as though failure of the plant to obtain an adequate water supply could be attributed to soil toxicity, but more often it is probably due to the fact that the clay soils with their high content of hydrophilic colloids are actually more retentive of water and the plant is unable to exert sufficient pull against these retaining forces. Ecologists are well aware that the percentage of non-available water in clay soils often reaches a high figure and that it is distinctly greater than that of soils more open in texture. Thus Gain(8) as far back as 1895 showed that with a mesophyte such as *Lupinus albus* the non-available water in clay was more than 11 per cent., whereas that in garden soil was nearly 3 per cent. and that in sand less than 1 per cent. The work of other plant physiologists from an even earlier date until the present confirms this fact.

It is not, therefore, a matter for surprise that on the very heavy clays of some parts of Trinidad the canes are extremely susceptible to a slight diminution in water supply and that in time of even mild drought they readily succumb to froghopper attack. Quite a small amount of

extra water might serve to turn the balance and enable the canes to make better way to recovery from the attack.

This point is well illustrated by an instance to which Williams (29) refers on p. 109. The Government Pond at Hermitage is situated by the roadside and to the south and south-west of it is an incline of 40 feet in 180 feet down to a footpath. The soil in the vicinity of the pond is a heavy brown clay and part of this field, coincident with the worst clay outcrop, was three years in succession damaged by froghopper. Extending down from the pond, where seepage supplied a slight extra amount of moisture, a strip of canes, approximately equal to the width of the pond, showed distinctly less damage.

While emphasising the importance of the water supplying power of the soil to the plant, which the writer believes is by far the most important bearing of the soil upon froghopper, it should not be overlooked that weakness of the cane may be due to other than water starvation. Soil toxicity has already been mentioned as a possible factor in the latter connection, but its toxic action may also affect the health of the plant directly. This requires much further investigation.

Again, the soil may be deficient in materials essential to cane growth. The neglect of crop rotation, which is almost without exception in the island, is bound to have the effect of depleting the soil of constituents necessary to cane. It is also noteworthy that old ratoons, which have for years drawn upon the soil in their immediate neighbourhood are much more subject to blight than young plants. Of course this is also largely attributable to an old and worn out root system. The great need for rotation of crops has been stressed by Williams and others and it deserves full attention.

In certain undulating lands such as occur in the south of the island there is an interplay of two or three soil types. Nearly all these soils are exceedingly heavy. There is some evidence, from the general contour of the land, that occasionally localised blight on land generally believed to be good is due to the presence of retentive subsoil pans which interfere with drainage. To test out this hypothesis a subsoil survey is necessary.

Effect of Fertilisers in Aiding Recovery.

With the knowledge that failure of cane under froghopper attack was due to loss of green leaf surface, and that the best method of aiding new leaf development was by providing a ready supply of soil water with required food materials, it was thought that fertilisers applied immediately after attack might have the desired effect. It was recognised

that addition of artificial manures such as nitrates and potash alone could have little or no effect in increasing availability of soil water and that they might, by increasing the osmotic pressure of the soil solution and by deflocculation, even decrease the availability. From the foregoing study available water was believed to be of first-class importance, and unless these added materials had also the effect of flocculating soil colloids or in other ways of reducing the retentiveness of the soil to water they might do more harm than good. For the purpose soluble phosphates seemed to be specially indicated on the heavy clay soils, and in addition it was thought that they might stimulate root growth. It has already been noted that canes upon compacted clays showed poor and shallow root systems.

Two series of experiments were made, with various manures, on blighted fields upon heavy clay. The second and larger series was very kindly undertaken by Mr G. A. Jones of the Usine Ste. Madeleine Sugar Estates. In both cases the control plots which had had no dressings gave higher yields of cane and better juice than the manured plots. These results will be given and discussed in a subsequent paper of the present series.

Effect of Rainfall.

Williams (29) has given lengthy consideration to the subject of rainfall and reference must be made to his papers. He shows that moderate rainfall for both wet and dry seasons is associated with decrease in the amount of blight, while excessive dryness or excessive rain for either season tends to favour increased blight. These views have been corroborated in the present study.

A wet-dry season favours some breeding of the froghopper. A wet spell in the dry season induces emergence of aestivating stages as adults and if this is followed by further dry weather damage becomes manifest on the canes, as has been described earlier in this paper.

In the wet season the froghopper breeds continuously and its numbers vary with natural controls. Given the presence of a large number of froghoppers, a slight dry spell will intensify the appearance of damage upon the canes very considerably by reducing the water available to the cane at this critical period.

Excessive rain in the wet season favours increase in the severity of blight. In addition to the effect of excessive rains upon the froghopper and the influences controlling it which Williams (29) has considered, I would draw attention to the effect of such upon the cane itself. Ex-

cessive rain on retentive soils is bound to cause temporary waterlogging with defective aeration which produces a result physiologically equivalent to water shortage. This effect has been frequently observed and needs no further elaboration.

Briggs and Shantz(4), p. 88, state: "The experiments made in connection with the effect of soil moisture content on the water requirement show, as a rule, an increase in the water requirement when the soil moisture content approaches either extreme." This further helps to explain the apparent contradiction that both drought and excessive rainfall have similar effects upon blight intensity.

Discussion and Conclusions.

Monecphora saccharina is a natural denizen of cane and grass fields when conditions are sufficiently moist and suitable for its breeding. Nevertheless canes do not necessarily show serious blight when froghoppers have been abundant, nor is abundance of the insect a necessary condition for serious blight. Of course a mass attack of enormous numbers of froghoppers cannot but have a seriously adverse effect upon canes, whether they be growing under good or bad conditions, and attacks of this nature do occur.

The foregoing study has indicated a few of the predisposing causes for increased intensity of the blight. It has not been an object here to attempt explanation of increase in actual numbers of the froghopper, although it is recognised that this has an essential bearing upon the problem. Williams has examined many sides of this question in detail and my own observations can add but little as yet to his careful enquiry.

It seems quite clear that at times the conditions favouring increase in numbers of froghoppers are correlated with those tending to increase susceptibility of the cane. Also susceptible canes sometimes appear to be actually more attractive to the insects. Whether this is really so or not it is impossible to say at present, but it is quite likely that cane leaves with subnormal water content contain carbohydrates in a less advanced stage of condensation and consequently more water-soluble and more easily available to the insect than those of normal cane leaves. Horn(10) has shown that the sucrose content of leaves increases with decreasing water content and at the same time starch decreases. Ahrns(1) further observes that sucrose and hexoses are products in a wilting leaf. That except for a small loss due to respiration the total sugar formed is represented by a corresponding reduction in starch content. That in presence of starch the sucrose content rises with falling

and falls with rising water content and this is true whether in darkness or in light. Sucrose content is to be regarded as a function of water content. Hexose content always rises in starch-containing leaves kept in the dark, but reaches a higher value in wilting leaves than in moist ones. Other investigators have recorded somewhat similar observations. As far as the cane itself is concerned I can offer no proof. Certain microchemical tests made by me were not at all conclusive although it is highly probable that in cane leaves the same rule holds good.

Other conditions favouring the froghopper may possibly be found in cane leaves with reduced amount of lignified tissue, thus allowing easier penetration of the insect's stylets. Such a suggestion does not appear to be borne out in fact. Also no variety appears to possess any appreciable degree of immunity from attack. Uba certainly stands up well under froghoppers, but it is equally subject to attack with other varieties.

Tannin content may have some effect upon the preference of the froghopper. Marañon (18) has shown that mildew resistance in *Oenothera* is associated with higher tannin and water-soluble acid content.

The acidity of the cane was thought possibly to have some influence upon preference of the froghopper, in view of the probability of an optimum *p*H zone for the activity of the insect's saliva. Again proof has not been forthcoming.

General observations indicate that the froghopper exhibits some, but no very marked preference among canes and that when increased numbers are found localised in fields of specially susceptible canes this is usually due to other causes as yet not fully understood.

The effect of the sucking of the froghopper upon the cane can be briefly outlined as a removal of water, carbohydrates, proteins, etc., from the plant, and an injection of enzymes, etc.

Withdrawal of water alone from the cells of the leaf results in a local water deficit which tends to be corrected if the osmotic pressure of the cell sap is increased such that water passes into the deficient cells from the surrounding tissues. Increase in osmotic pressure of the cell sap is, however, commonly effected by hydrolysis of carbohydrates and the decomposition of other complex molecules to simpler structures, and as rapidly as these hydrolytic products are formed they are removed by the insect, whose saliva is also assisting in the decomposition processes. It is clear that a state can be reached in which the physiological equilibrium of the plant cell has been so far thrown over that recovery, even with ample and immediate water supply, is impossible.

Ullrich (25) working with *Zea* has shown that only such chloroplasts as have lost relatively little protein, by nitrogen starvation, are capable of renewed protein formation. The froghopper undoubtedly removes proteins among other products from the chloroplasts and this may be responsible for death of the chloroplasts from which removal of carbohydrates is possibly not so serious a matter. Chloroplasts, as also other protoplasmic structures, may of course be killed directly by the toxins presumed to be present in the saliva of the insect if they are sufficiently exposed to their action.

The almost specific and primary effects which have been noticed upon the border parenchyma cannot be without effect upon the translocation of plant food materials, and it would seem more than likely that the very necessary local water supply should be interfered with.

The effect on the phloem and on other vascular tissues which cannot be detected immediately is probably very important and far reaching. In a day or two the phloem begins to show blocking and is obviously disorganised in function. Such a state cannot fail to impede the passage of water, etc., to and from the neighbouring tissues. Meanwhile, the enzymes of the froghopper's saliva and the released plant enzymes continue their action and spread from one water-deprived cell to another. Extending death of the tissues is the consequence until cells capable of reacting successfully are able to arrest further progress of the pathological changes.

Increase in transpiration may occur locally after puncture. It has been demonstrated on a few occasions but continuance of the increase is doubtful. There is certainly no immediate reduction in transpiration in the vicinity of injury. Reduction in transpiration rate would, by retaining the cell water, probably assist materially in local recovery.

Oxidases of the plant and of the froghopper's saliva seem to be responsible for colour changes and the production of red pigment, but more important is their effect upon the H-ion concentration of the cell sap. It is almost certain that increased acidity results directly from oxidation. Increased acidity may quite probably bring the tissues of the phloem, border parenchyma, and chlorenchyma nearer the optimum for the insect's salivary enzymes and also for those of the plant which have been released. Sherman (23) has shown that the zone of optimum activity for malt diastase lies between *p*H 4·3 and 4·5, that is in the isoelectric zone of the enzyme itself. This enzyme, which is a protein, therefore exerts its greatest effect upon starch when it is least hydrated and least ionised. It may well be that the diastase of the froghopper's

saliva has an optimum zone very near that of malt diastase, in which case one would expect increased acidity of the above-mentioned tissues to favour the enzyme action. Be this as it may, a lowering of the *p*H of the cell sap of the cane is a marked pathological symptom and canes which show but slight lowering usually recover. A rapid and considerable drop is associated with canes likely to succumb. It therefore seems reasonable to expect that for a cane to recover it must resist the change in *p*H or in other ways arrest continued autolytic enzyme action. H-ion concentration could be stabilised by buffering agents in the cell sap. Uba cane, which stands up against the effects of froghopper better than any other variety is noted for the higher colloid content of its juice. May not certain of these colloids, especially the albumins, exert a beneficial buffering effect? It is more than probable that they do so. Again, tannins are known to have an inhibitory effect upon enzymes and they may be important anti-enzymes in the cane. Decreased susceptibility to froghopper may be inseparable from increased colloid content of the cane sap, and should this prove to be true a good cane for froghopper conditions will probably be a poor cane from the viewpoint of the sugar manufacturer. Various authors have shown that drought and frost resistance in plants may be associated with increased hydrophilic colloid content of the sap, particularly the pentosans. *Vide* Newton(19) and bibliography to this paper.

The loss of active leaf surface resulting from froghopper injury may be very considerable and, as has often been remarked, the canes present appearance such as if a fire had passed through them. The reduction of photosynthesising surface must result directly in a corresponding reduction in the supply of elaborated products. This accounts for the small starved internodes of the stem which are laid down during the course of blight. Further, the pathological changes already noted in the leaf may continue in the stem from the top downwards; the top being itself richer in oxidases is the first to show a rise in H-ion concentration. With rise in H-ion concentration and continued enzyme activity carbohyrates are hydrolysed in the stem and it is likely that the increased osmotic concentration of the cell sap thereby produced is responsible for the shooting of the eyes lower down the stem—another feature of blighted canes. This of course occurs especially if the terminal bud has died.

Recovery of the stem is only possible with renewed leaf surface and renewed power of photosynthesis. This necessitates ample water with required solutes from the soil. The soil may be, and often is, on badly

blighted fields, physically or physiologically retentive of its water so far as the plant is concerned; heavy clays and badly aerated or toxic soils being in mind. Fertilisers have not been found beneficial at this critical time. They may possibly increase the osmotic pressure of the soil solution by a small fraction, but none the less sufficient to affect a struggling cane. This point, however, requires investigation. The explanation offered here is not too probable.

Suggestions for Consideration and Trial, Based upon the Foregoing Study.

I. Cane varieties most suitable to the soil conditions should be grown. There is however little choice. Canes likely to withstand froghopper attack best are such as can obtain and maintain a water content well above the wilting point under adverse conditions. In this connection it should be remembered that the water requirement of plants upon unfertile soils is greater than that of those upon fertile ones, as Kiesselbach has shown (15), although the *total* water necessary is more on the fertile soil, due to increased growth. The more xerophytic types of cane such as Uba suggest themselves as more suitable for employment upon bad soils. Uba plants used as supplies among seedling varieties stand up markedly in blighted fields when the seedlings have been annihilated. No freedom from froghopper attack can be claimed for Uba, merely greater vigour under adverse conditions and this is rather offset by its less desirable factory qualities, which may be directly associated with its drought and other resistance. Agaul might be tried, as it is stated to be resistant to drought and higher in sucrose content than Uba (31).

II. As far as possible a high water content should be maintained in the cane plants. Experimentally some promising results have been obtained by decreasing transpiration, but field tests have thus far been inconclusive. A slower transpiration stream may be undesirable if reduced transpiration results in reduced solute intake by the roots. As field confirmation is not yet forthcoming the two following suggestions are not put forward with confidence, although it is intended to continue further along these lines: (1) maintenance of a humid atmosphere around the leaves, to reduce evaporation, and (2) shading from light, to reduce transpiration as such. Wind breaks might be indicated, but they are unlikely to be of practical value. Blight on the tops of ridges in dry weather may sometimes be intensified by wind passage. As regards shading, this again is a matter for practice to decide. The planting of

shade trees with cane is never done; it would increase difficulty of reaping and if too much light were cut off the sucrose content of the canes would undoubtedly suffer. An optimum shade could only be determined experimentally. The temporary erection of light shade over blighted canes in the field has given no definite results, but the period was exceptionally wet during these experiments. It is hoped to repeat them in dryer weather.

III. An endeavour should be made to increase the available water in the soil. Water may be unavailable to the cane (1) from actual deficiency or (2) from physiological unavailability. Actual deficiency of water may occur during the wet season, especially in time of the Indian summer or *petit carême*. A dry spell during the wet season while froghoppers are abundant is disastrous, especially upon infertile soils, where plants exhibit a greater water requirement. Physiological unavailability of water may result from many conditions. (1) High content of hydrophilic colloids, such as is found in very heavy clay soils. This is noteworthy in several districts of Trinidad. (2) Oxygen deficiency in the soil. This again obtains especially on heavy compacted clay soils in bad state of tilth. They have a lower oxygen content than more open soils even under the same rainfall conditions. Livingston and Free state that interference with water absorption by the roots is the first result of reduction of oxygen content of the soil. Roots die with prolonged lack of oxygen and drought following upon this is dangerous. Waterlogged soils again are lacking in aeration. Severe blight has been noted on several occasions as a result of waterlogging and defective drainage. There are indications occasionally suggesting that an impermeable subsoil pan may be interfering with drainage and that such may be responsible for very local intensification of damage. This might be very difficult to rectify. (3) Soil toxicity of various kinds, inorganic and organic. Root development and intake of water is interfered with on toxic soils. (4) A poor root system. Canes on compacted or toxic soils often have ill-developed roots. The same applies to canes affected with root disease and it is probable that nymphs of froghoppers feeding upon the roots have a deleterious effect upon root development. All these factors may be interdependent. Fertilisers applied at the surface may sometimes have a bad effect by inducing a superficial root formation which is dangerous in time of drought. (5) High soil temperature. In certain rare cases of blight following upon weeding and trashing of the canes (which may be done by planters in the hope of reducing the numbers of froghoppers at the last moment) it is possible that greater

exposure of the soil to the rays of the sun, particularly at midday, may produce a soil temperature sufficiently high to retard root absorption at a time when water is most needed by the leaves. (6) High osmotic concentration of the soil solution. Such is unlikely on ordinary soils, but it is a possibility to be borne in mind.

From the most cursory observation it is clear that usually many factors operate at once and it is for this reason that evidence often appears conflicting.

An obvious recommendation falling under the present heading of increasing water availability is one which both Nowell and Williams particularly have emphasised, namely the improvement of the physical condition of the soil by thorough tillage and attention to general agricultural practice. Liming is probably more valuable under this heading than in its capacity for correcting soil acidity. Phosphate applications would seem to be indicated on many heavy clays both for their flocculating powers, for correction of possible aluminium toxicity and for stimulating root development, but experimental results have not been promising. The matter will be dealt with in a later paper. On the other hand one would do well to avoid fertilisers known to have a peptising effect upon the soil.

One great desideratum in Trinidad is a crop, preferably leguminous, which can be grown in rotation with cane, to be used as a green dressing. It should be a good cover crop, to keep down weeds, and when turned into the soil should be sufficiently woody to decay slowly and leave air channels, thus assisting in aeration of the soil.

Irrigation is not practicable in most parts of Trinidad and in any case it would be troublesome and difficult to regulate upon heavy clays. It might do more harm than good by reducing soil aeration. It would be costly to establish. Trinidad has a heavy rainfall and an excess rather than a scarcity of water during the greater part of the wet season. Irrigation during the dry season would no doubt induce the froghopper to continue breeding throughout the year. Flooding to kill the nymphs is useless as they ascend the stems with a rise in water level.

Several other sucking insect pests have been noted as being influenced by environmental conditions and highly commendable research is that of Andrews (2) upon the Tea Mosquito Bug, *Helopeltis*. He shows that "an approach to waterlogged conditions and an increasing soil acidity conduces to a greater liability to suffer from serious attack," but he lays most stress upon the ratio of available (not total) potash to phosphorus in the soil. Similarly he finds that the amount of potash

compared with that of phosphoric acid in the leaf is correlated with degree of immunity. Finally he clinches his arguments by stating that when a constant supply of soluble potash is applied to attacked plants they can throw off the attack completely and remain immune for the rest of the season.

Response to manuring with potash has not been obtained with froghopper blight and no analyses have been made of the leaves. Similar influences may be at work in both tea mosquito and froghopper blights. Potash may be necessary in Trinidad but unavailable due to some interfering factor. Further work needs to be done along these lines.

There is a possibility that the relative abundance of various species of thrips is dependent upon the water relations of their host plants. When in England, some years ago, the writer had occasion to observe a case of thrips attack upon *Fuchsia*, under greenhouse conditions, following upon reduction of water, while similar plants regularly watered were not attacked. Recommencement of regular watering resulted in a gradual disappearance of the insects and a final freedom of the plant. Dr S. C. Harland tells me that he has observed another such case at the Shirley Institute, Didsbury. Cotton plants were being grown with varying water supplies. Thrips attacked only those plants which were short of water.

In the West Indies and elsewhere *Kakothrips rubrocinctus* seems to behave similarly upon cocoa. The pest often occurs on the edges of the plantations where the atmosphere around the leaves is presumably less humid or else it commonly becomes a nuisance in the dry season. Such is the rule. Ballou (3), quoting an exception to the dry season incidence of thrips, in Grenada, says "I may say that whenever I was shown thrips attacks, I found root disease, or insufficient drainage, or very shallow soil with terrace or heavy clay beneath, or lack of humus." "I was shown several instances where trees had been freed from the insects by drainage." Thus again we probably have a state of physiological water shortage, probably associated with reduced foliar transpiration; conditions favourable to the insect.

Wilkinson (28) remarks that *Diarthrothrips coffeae* is unable to live on shaded coffee trees in Uganda. The shade no doubt is responsible for increased atmospheric humidity, and reduction in transpiration probably preserves a higher and more equable water content of the foliar tissues of the coffee trees.

Other examples, giving similar indications could be quoted and I think that one may say that there is fair evidence of the water relations

of plants being responsible for the prevalence or otherwise of several sucking insect pests.

The importance of the availability of plant food materials other than water has not been overlooked, but the availability of these is inextricably interrelated with the availability of water. All food materials, minerals, etc., must be taken up in solution by the roots. Water is the vehicle. With further research we may reach deeper. This is passing beyond the work of an entomologist, indeed it is with much trepidation that the writer has ventured so far, but it is hoped that further work by those competent will complete our evidence on this most important phase of insect control.

Acknowledgements.

Work of such nature as the present has necessarily brought me into association with many agricultural interests and everywhere the most willing assistance has been offered. My best thanks are due to all. I am under particular obligation to Mr W. Nowell of the Trinidad Department of Agriculture and also to Mr F. W. Urich of the same Department. Mr G. A. Jones of the Usine Ste. Madeleine Estates has been ever willing to carry out suggested experiments for me, however unlikely of commercial success. Messsrs Grant of Caroni Estate and Taylor of Orange Grove Estate have also afforded me kind assistance. The demands which I have made upon my colleagues at the Imperial College of Tropical Agriculture have been frequent in every department and to them all, by no means least, do I tender my cordial thanks.

Finally may I emphasise the fact that an enormous amount of work has been saved me by the excellent report of Mr C. B. Williams (29)? Familiarity with his report is assumed throughout this paper and confirmatory data for many opinions expressed by me are to be obtained by reference to his work.

Summary.

(1) The froghopper (*Monecphora saccharina* Dist.) is a serious pest of sugar-cane in Trinidad.

(2) Of the two important aspects of the problem namely (1) the factors controlling the numbers of the froghoppers themselves, and (2) the conditions influencing resistance of the canes to, and recovery from attack, the second is considered in the present paper.

(3) The bionomics of the froghopper are briefly dealt with.

(4) The saliva of the froghopper is slightly acid (*p*H 6·0–6·2) and it contains amylytic and oxidising enzymes.

(5) The effect of the froghopper sucking the cane leaf is described, noting especially the peculiar and primary effects upon the border parenchyma.

(6) Later spread of injury is described in detail and various influences at work are considered.

(7) The water relations of the plant appear to be highly important in connection with recovery from injury and retardation of the spread of injurious effects.

(8) Fertilisers have not been shown to aid recovery.

(9) The problem is discussed in several of its aspects and the contents of the paper, with suggestions for further investigation, summarised on pp. 102–106. "Canes do not necessarily show serious blight when froghoppers have been abundant, nor is abundance of the insect a necessary condition for serious blight." A *great* abundance of froghoppers, of course, is bound to have a seriously deleterious effect, quite apart from the condition of the cane. The importance of water relations is emphasised.

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EXPLANATION OF TEXT-FIGURES

Fig. 1. Transverse section of Uba cane leaf, near middle, to show distinctive tissues. × 300.
Fig. 2. Longitudinal section of D. 625 cane leaf. × 300.
Fig. 3. Longitudinal section of *Paspalum* grass leaf, through a froghopper puncture. × 300.
Fig. 4. Longitudinal section of *Paspalum* grass leaf, through vein next to the one actually pierced by a froghopper and sucked for five minutes. Fixed at once. Note salivary effect extending longitudinally from cross veins (*cv*). Affected cells shown with paler plastids, unaffected cells shown black. Chlorenchyma omitted for clarity. Drawn from a section stained with safranin O and picro aniline blue. × 167.

All drawings made with an Abbé camera lucida.

LETTERING.

bp, border parenchyma; *chl*, chlorenchyma; *cv*, cross-vein; *ep*, epidermis; *mt*, motor tissue; *ph*, phloem; *scl*, sclerenchyma; *ss*, stylet sheath; *st*, stoma; *xy*, xylem.

(*Received October 20th*, 1925.)

A COMPARATIVE STUDY OF THE FEEDING METHODS OF CERTAIN HEMIPTERA AND OF THE RESULTING EFFECTS UPON THE PLANT TISSUE, WITH SPECIAL REFERENCE TO THE POTATO PLANT

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(With Plates I–IV and 10 Text-figures.)

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Introduction.

This study was first commenced in 1921 when the writer was engaged upon some experiments in connection with the transmission of the so-called "virus diseases" of the potato by means of aphides and allied sucking insects.

It has now been fairly conclusively proved that one of the chief agents in the spread of virus diseases, not only of the potato but of many other plants, is one or more of the species of aphides attacking those plants. The evidence does not yet seem sufficient to incriminate, as vectors of potato mosaic, the other sucking insects such as leaf-hoppers and Capsid bugs which also habitually live upon the potato plant, although it tends to point in that direction.

Another point which has a direct bearing upon the present study is the fact that not every species of aphis seems able to act as a carrier of virus disease. On the facts as we know them so far, it appears strange

that this should be so. Why should not every species of aphis or for that matter every sucking insect act as a transmitting agent?—always supposing in the first place that the insect is a mechanical carrier of the disease and does not act as an alternate or obligate host for the pathogene.

It was then in the hope that a detailed study of the feeding methods of plant-sucking insects, together with a study of the effect of such feeding upon the tissues of the host, might throw some light upon the question as to what insects can or cannot spread the disease, that this work was undertaken. In this paper, therefore, attention is directed more to the study of the above problems than to the question of virus disease. Some further experimental work is now proceeding on such disease transmission by insects, and it is hoped to correlate later the results of the two studies.

Several workers, among whom may be mentioned Busgen(5), Petri(16) and Davidson(6), have studied the feeding of various aphides upon different plant hosts. So far as the writer is aware however, no one has yet made a comparative study of the feeding methods of the Hemiptera as a whole.

Acknowledgements.

Acknowledgements are due to Dr Davidson of the Rothamsted Experimental Station and to Dr W. Robinson of the Botanical Department of this University for suggestions and criticisms; also to Mr H. Britten of the Manchester Museum for suggestions in regard to photomicrography.

Technique.

The insects were studied *in situ* upon the leaf or stem of the host, and both fresh and fixed preparations were made. The fixing fluids used were the following:

Carnoy's fluid, No. 2 *Formula* (both hot and cold). Glacial acetic acid, one part; absolute alcohol, six parts; chloroform, three parts.

Farmer's fluid. Absolute alcohol, two parts; glacial acetic acid, one part.

Corrosive sublimate (hot). Corrosive sublimate, 3 gm.; glacial acetic acid, 3 gm.; alcohol (60 per cent.), 100 c.c.

Flemming's fluid (weak). 1 per cent. chromic acid, 25 c.c.; 1 per cent. acetic acid, 10 c.c.; 1 per cent. osmic acid, 10 c.c.; water 55 c.c.

Carnoy and Flemming gave the best results, the latter gave especially good fixation with such firmly attached insects as aleyrodes, and Coccidae and with fixing plant tissue alone.

Little difficulty was experienced in fixing aphides and aleyrodes *in situ* in the tissue, but with such large and lively insects as leaf-hoppers, etc., a special technique had to be devised.

The following method was evolved for dealing with the leaf-hopper. A leaf covered with the undisturbed insects was placed near a dish containing hot Carnoy. Small drops of molten wax were allowed to fall upon individual insects, as little wax being used as possible. The whole leaf was then plunged into the hot Carnoy, and allowed to remain a few minutes. A small square of leaf containing the insect and the drop of wax was then cut out and allowed to fix for 24 hours in the fluid.

The temperature of the molten wax was sufficient to kill the insect instantaneously, and its consistency prevented any withdrawal of the stylets. The chloroform in the Carnoy and subsequent technique dissolved away the wax covering, leaving the insect fixed *in situ*. This method was used for the larger nymphs only; a sufficient number of the smaller stages were fixed *in situ* using hot Carnoy alone.

The preparations were stained in various ways. For stylet tracks of the aphides, safranin in 70 per cent. alcohol was used, either alone or as part of Flemming's triple stain (safranin, in 70 per cent. alcohol, gentian violet in clove oil and orange G in clove oil). Heidenheim's iron haematoxylin and Delafield's haematoxylin also gave good results.

All the drawings were made with the aid of a camera lucida, using a 1/12th oil immersion objective and a No. 2 or No. 4 ocular of Zeiss.

Scope of the Work.

It does not lie within the scope of these studies to give a detailed description of the mouthparts and the mechanism of suction in the Hemiptera. For this the reader is referred to the following workers who have published detailed accounts: in the Aphidae Davidson (7), in the Capsidae Awati (1), in the Aleyrodidae Hargreaves (12), also Grove (11), Berlese (3) and others.

Efforts have been made to elucidate the following points in connection with the species studied. It is hoped that some correlation between such points and ability or otherwise of the insect to transmit virus disease may transpire.

(*a*) Particular leaf-face attacked, *i.e.* upper or lower surface.
(*b*) Position of the insect upon such leaf-face in relation to the veins.
(*c*) Point of entry of stylets.
(*d*) Path of stylets in the tissue, whether inter- or intra-cellular.

(*e*) Objective of stylets.
(*f*) Effect of the penetration and saliva upon the plant tissue.
(*g*) Method of penetration of tissue.

Insects Examined.

The following insects were used in the investigations and are dealt with in their order of classification.

HEMIPTERA.
Heteroptera.

CAPSIDAE.	*Calocoris bipunctatus* Fab.
	Lygus pabulinus Linn.
Homoptera.	
TYPHLOCYBIDAE.	*Eupteryx auratus* Liv.
	Zygina pallidifrons Edwards.
ALEYRODIDAE.	*Asterochiton vaporariorum* Westw.
APHIDIDAE.	*Myzus persicae* Sulzer.
	Macrosiphum solanifolii Ashmead.
	Myzus circumflexus Buckton.
COCCIDAE.	*Aspidiotus hederae* Vallot.
	Dactylopius longispinus Targ.-Tozz.

Of these groups of Hemiptera the Capsidae, Typhlocybidae and Aphididae are among the normal insect fauna of the potato. *Asterochiton vaporariorum*, the well-known greenhouse white fly, attacked potatoes which were being grown experimentally in its vicinity and so was included in the studies. The Coccidae were studied for the sake of comparison and because the group is thought to be capable of disseminating virus disease (Elmer (9)).

The species of aphis *Myzus circumflexus* is not a usual inhabitant of the potato plant though it takes readily to it. It was included as efforts had previously been made without success to induce it to act as a carrier of mosaic disease and a comparison of its feeding methods was desired with those of the true potato aphides *Myzus persicae* and *Macrosiphum solanifolii*.

Detailed Study of the Species.

HEMIPTERA.
Heteroptera.

CAPSIDAE. *Calocoris bipunctatus* Fab.
Lygus pabulinus Linn.

The following descriptions of both insects are taken from Saunders (17).

"*Calocoris bipunctatus*. Green, clothed with rather thick black hairs, pronotum generally with two small black spots on the disc, elytra in

the male sometimes more or less reddish brown between the nerves, membrane dusky, nervures pale, femora more or less freckled with brown. Basal joint of antennae scarcely thickened, third and fourth slenderer than the second, together about equalling it in length, pronotum shining, much raised posteriorly, with a narrow raised collar, sides nearly straight, surface slightly wrinkled transversely, elytra with the sides slightly rounded, posterior femora slightly incrassated. Length 7 mm."

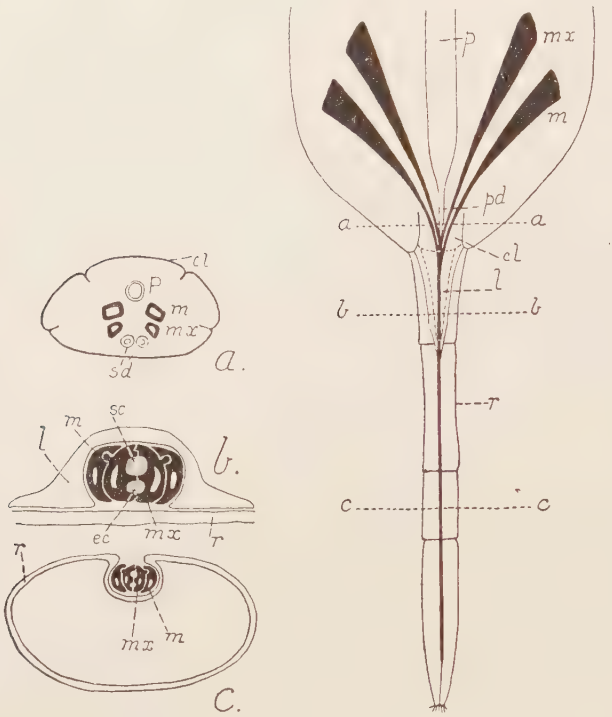
"*Lygus pabulinus*. Green, elongate oval, slightly shining, punctured, clothed with pale hairs; antennae with the apex of the second joint and the third and fourth joints black, basal joint about as long as the head, third joint about three quarters as long as the second, fourth about three quarters as long as the third; pronotum with a narrow raised collar, sides nearly straight, base rounded, anterior callosities strongly developed; scutellum transversely rugose; elytra elongate, their sides slightly curved, membrane with a longitudinal cloud below the apex of the cells; femora elongate, tibiae with very fine, scarcely noticeable bristles. Length 6 mm."

These two capsid bugs are very common insects, and, though not confined in their attacks to the potato, occur very frequently upon that plant. Of the two, *C. bipunctatus* is the more truly known as the "Potato Bug." It will be advantageous here to give a short description of the mouthparts of the capsid bug; such description with modifications is true for the Hemiptera as a whole and will apply to the other members of the group here studied.

The general structure of the mouthparts in the whole group of the Hemiptera is very similar, practically all the insects excepting some carnivorous species feeding in the same manner, *i.e.* by sucking the juices of the host plant. The mouthparts are therefore specially adapted and consist of a grooved labium long, except in the Coccidae, in which lie the mandibles and maxillae. These two latter form a single piercing and sucking organ, and it is this organ which is inserted into the plant; the labium itself never penetrates the plant tissue. The actual piercing organ then consists of two pairs of hollow needle-like structures, the outer pair being the mandibles and the inner pair the maxillae. In the Capsidae and some Jassidae the mandibles are barbed at their extremities, the maxillae are never barbed but are smooth and taper to a fine point. Each maxillary stylet is grooved along its inner side and each groove is divided by a ridge into two parallel grooves. By the apposition of the maxillary stylets two fine channels are formed, down

one of which passes the salivary secretion of the insect and up the other the sap of the plant. These mouthparts, therefore, function with the pharynx as a sucking apparatus and as a saliva injector worked by the salivary pump situated in the head.

Text-fig. 1 is an explanatory diagram of the mouthparts of the



Text-fig. 1. Diagram of the mouthparts and adjacent region of the head of an hemipterous insect. On the left are transverse sections across the regions bearing corresponding lettering; the magnifications of these sections are not uniform. *cl*, clypeus; *ec*, ejection canal with salivary duct; *l*, labrum; *m*, mandible; *mx*, maxilla; *p*, pharynx; *pd*, pharyngeal duct; *r*, rostrum; *sd*, salivary ducts; *sc*, suction canal with pharyngeal duct. [After Imms, *General Textbook of Entomology.*]

Hemiptera. The capsid feeds upon either the upper or lower surface of the leaf apparently without particular preference. It confines its attention very largely to the young leaves and shoots of the plant, and occasionally attacks the stems as well. The insect appears to select the actual point of penetration in the leaf with great care, feeling the surface with the tactile hairs at the end of the labium. Certain specimens under

observation probed the leaf surface with the proboscis for five or six minutes before penetrating the tissue.

As shown by these observations, the capsid plunges the stylets into the leaf or stem and withdraws them several times in a very circumscribed area.

Text-fig. 2. Section of potato stem showing track of ovipositor of *Calocoris bipunctatus*. ×950.

The stylets enter the tissue of the leaf either inter- or intra-cellularly, the cell wall offering little resistance to the considerable pressure exerted by such a large insect.

The stylets follow no particular path as the capsid is able to pierce any part of the tissue at will, but in consequence of this the track of the stylets becomes mainly intra-cellular.

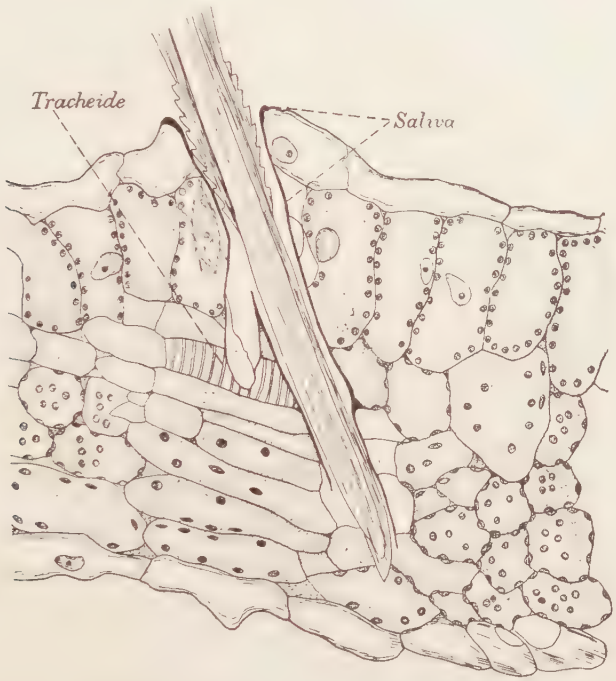
This insect appears to tap any part of the tissue and does not, like the aphides, confine its attention to the vascular bundles, which, however, it does tap on occasion. Awati (1) figures the stylets of *Lygus pabulinus in situ* in the xylem vessels of the host plant.

The purely mechanical damage done by both species of capsid bug to the tissues of the potato plant is quite considerable and is due to two causes. Firstly, to the serrated edge of the mandibular stylets (also found in some Typhlocybidae, *vide* Text-fig. 3), which are fitted with backwardly directed barbs, and secondly to the habit previously mentioned, which the capsid has of frequently withdrawing the stylets and plunging them again into almost the same spot in the plant tissue.

That the saliva of some capsid bugs contains a principle which is violently toxic to plant life has previously been demonstrated by the writer (18). The effect of the feeding of the two species of capsid bugs upon the tissue of the potato plant or for that matter upon any plant host is very great and exceeds in severity the effect produced by any of the other members of the Hemiptera. Wherever the plant has been punctured by the stylets of either bug, the tissue surrounding the puncture for a considerable distance is killed. In the case of a severe attack the effect of such puncturing upon the health of the potato plant is marked. The young shoots are killed and the leaves are covered with brown patches which later fall out, giving a "shot-hole effect," *vide* Horne and Lefroy (13).

In order to study microscopically the effect of the injections of the capsids upon the tissue of the potato plant, leaves punctured by the insects were fixed after varying periods had elapsed from the time of puncturing. Plate I, figs. 1, 2, 3 show photomicrographs of punctures in leaves fixed four, twelve and twenty-four hours respectively after feeding. Fig. 1 shows a puncture from the lower epidermis; it will be seen that the stylets have penetrated almost the entire thickness of the leaf. A large area of the epidermis is already destroyed, and the effect of the saliva may be seen spreading to right and left of the original puncture. As yet however most of the leaf tissue is unaffected. Fig. 2, part of a leaf fixed twelve hours after being punctured, shows the destruction further developed. Holes are forming in the tissue and the epidermis of the lower surface has now degenerated into a thickened layer of horny substance. Fig. 3 shows the final stage in this disintegration of the leaf.

The injury is now represented by a sunken area of brown material in which all resemblance to plant tissue has completely disappeared. Fig. 4 is a photograph of a section taken outside the actual area of the puncture; it shows the spreading of the salivary material inside the tissue of the leaf into the vascular bundles. Three tracheides may be seen blocked up with the salivary injection. It is interesting to compare Fig. 5 which shows the track of the proboscis in the tissue with Text-fig. 2 which shows the track of the ovipositor (*vide* Smith (19)) in the stem. In the

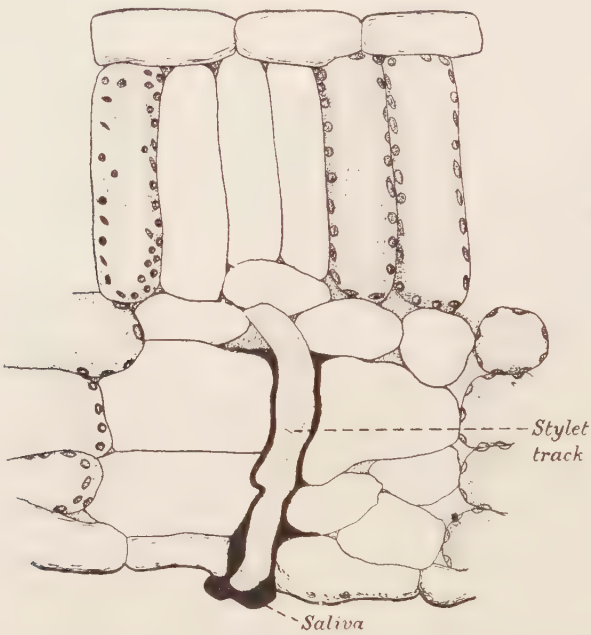


Text-fig. 3. Stylets of leaf-hopper *in situ* in potato leaf. Note the barbed mandibles and the tapping of the tracheide. ×950.

former the pathological effects of the accompanying saliva are plainly seen, while in the latter there is no more injury than a mere physical tearing of the cells. An exactly similar effect was produced by puncturing the tissue with a sterile needle.

In examining capsid punctures in the tissue of the potato leaf, the cells lying in the injured area are found to be blocked up with a granular substance which, like the stylet tracks of the aphis, very readily picks up safranin stain. The question arises as to what is the nature of this substance. Is it the salivary secretion of the insect alone or is it formed

by a reaction on the part of the plant to the secretion? It seems probable that it is a combination of saliva, cell sap, substances produced by reaction between the two, and the disintegrated remains of some of the cell walls. In earlier experiments by the writer(18) on another species of capsid bug on a different host plant, unsuccessful attempts were made to produce this granular substance artificially by means of inoculations with various poisons, and also by means of mechanical lacerations. As will be shown in the discussion of the aphides in a later section of this paper the "stylet sheath" formed in the plant by the feeding of various



Text-fig. 4. Puncture by leaf-hopper through lower epidermis of potato leaf. Note the stylet track and destruction of chloroplasts. ×950.

aphides is thought to be composed of substances produced by the reaction of the saliva on the cell sap. It seems then reasonable to consider the mass of material produced by the capsid punctures in the plant tissue to be similar in nature to that lining the stylet tracks of the aphides. The difference in the amount of the material formed by the capsid can be put down partly to the larger quantity of saliva injected and partly to the greater toxic powers of such saliva.

As would be expected of a comparatively large insect like the capsid bug, the stylets are driven into the plant tissue by mechanical force

although the penetration is almost certainly assisted by the dissolving effect upon the cell walls of the accompanying salivary secretions. In feeding the proboscis bends and thereby lengthens the stylets. Awati(1), p. 728, gives a detailed description of the mechanism of this process in *Lygus pabulinus*.

In feeding, then, the stylets of these insects follow a path in the tissue which is mainly intra-cellular. The evidence shows that the phloem is not the only objective of the insect but that any part of the tissue is tapped. The plant is penetrated by pressure. There is no stylet track comparable to that of the aphis, the toxic nature of the saliva being such that the puncture is marked by holes in the tissue, surrounded by masses of dead and dying cells filled with the salivary material.

HEMIPTERA. Homoptera. jassidae.

typhlocybidae. Leaf-hoppers. Species examined: *Eupteryx auratus* Liv.
Zygina pallidifrons Edw.

The next insects to be considered are the Typhlocybidae or Leaf-Hoppers. The two species used were *E. auratus* and *Z. pallidifrons*; the first is a normal and abundant inhabitant of the potato plant and the second common in many greenhouses fed readily upon the potato plant under experimental conditions.

The following description of the group Typhlocybidae is taken from Edwards(8), p. 209: "Small, elongate species, for the most part brightly coloured. Ocelli not, or scarcely, perceptible, sometimes indicated on the forehead. Face very long, triangular. Elytra much longer than the abdomen, overlapping at the apex; appendix wanting (except in *Alebra*). Corium with three simple longitudinal veins only. The latter character distinguishes this group from all others."

The Jassidae have been shown by various workers, among them L. O. Kunkel, to carry diseases of plants. Perhaps the best known example is *Eutettix tenella*, the beet leaf-hopper, which transmits the disease of beet known as Curly Top. Kunkel(15) finds that *Peregrinus maidis*, a species of leaf-hopper, was capable of acting as a carrier of mosaic disease in the case of corn.

In their feeding the Jassidae produce in the leaves of the plant host a white spot round each puncture which, so far as the writer is aware, is typical of the whole group. This white spot is produced whatever may be the host plant and does not vary in colour on different hosts as do the spots produced by capsid bugs; *vide* Smith(18), p. 53.

The leaf-hopper when feeding attacks the leaf from either the upper or lower surface with a preference for the under surface. This is especially so in the larval stages. It does not, unlike the foregoing capsid, appear ever to puncture the stem. There were no indications to show that it punctured particularly in the neighbourhood of a vein, and this is borne out by the subsequent microscopic examination.

The stylets of the leaf-hopper, the mandibles of which are barbed, *vide* Text-fig. 3, enter the leaf indiscriminately. The comparatively strong stylets are able to pierce the cell walls of the epidermis with ease. Plate II, fig. 1, is a photomicrograph taken of the leaf-hopper (*Zygina pallidifrons*) in the act of piercing the lower epidermis of the leaf. The breaking of the cuticle and underlying cell walls before the pressure of the stylets is well shown. There is a slight depression formed upon the leaf-face before the insect penetrates, similar to that made by a pin when pressed into a sheet of paper. Penetration therefore by this insect is made by pressure alone.

From the manner of entry of the stylets it will be understood that the path followed in the tissue will be mainly intra-cellular, the cells being penetrated at will.

Text-fig. 3 is a drawing of the stylets of a leaf-hopper *in situ*, entering the leaf in this case from the upper epidermis. The stylets have entered at an oblique angle and traverse practically the whole thickness of the leaf blade.

The objective of this insect when feeding appears to be twofold, firstly the palisade cells and secondly the vascular bundles. In Plate II, fig. 2, the stylets can be seen tapping the palisade cells, it is in this tissue that the effect of the punctures is most evident (Plate II, fig. 4). That the vascular bundles are also sought for by the leaf-hopper is clearly shown in Plate II, fig. 3, where the stylets can be seen making directly for a tracheide. This is demonstrated again in the drawing, Text-fig. 3, where a tracheide has been tapped and the stylets afterwards withdrawn.

The effect of the feeding by both these leaf-hoppers upon the tissue of the potato leaf is very marked. Externally the punctures show themselves in the white spot previously mentioned and which is typical of the whole group. Owing to the barbed mandibles of the jassid, *vide* Text-fig. 3, there is mechanical laceration to a slight degree at the point of entry into the tissue. The stylets, which are large compared to those of the aphis, leave a broad track across the tissue of the leaf when withdrawn, *vide* Text-fig. 4.

In Plate II, fig. 4, may be seen a photomicrograph of a transverse section of a potato leaf which has been punctured by *Z. pallidifrons*. The section passes through one of the white spots produced by these insects. The affected area can be seen sharply marked off from the normal tissue owing to the destruction of the chlorophyll. The cell walls are still intact but the contents of the cells have entirely disappeared. Under high magnification it is possible to see one or two now colourless plastids within the affected cells.

The salivary secretions of the leaf-hopper presumably diffuse through the cell walls without injuring them and decolorise and destroy the chlorophyll. The spots enlarge somewhat after the insect has moved away showing that the secretion continues to spread within the tissue. This destruction of the chlorophyll and the production of apparently intact empty cells is also occasionally produced in a slight degree by the aphis (*vide M. persicae*, Text-fig. 7) but the white spots are not easily seen as are those produced by the jassid, probably because they are so much smaller, consisting as a rule of only one or two cells. Horne and Lefroy (13), p. 370, suggest that this white appearance may be in part an optical effect due to included air; probably however the destruction of the chlorophyll is the true explanation of the formation of the white spot, and accounts for the fact that the spot is invariably white, whatever be the plant host.

By growing potatoes under glass cylinders under conditions of great heat and humidity it was found that these spots caused by the leaf-hopper increased to a great extent. The white spots under these conditions enlarged considerably, turned brown and finally dropped out of the leaf altogether. Fenton and Hartzell (10) in America describe a somewhat similar injury to potato plants produced by a leaf-hopper, *Empoasca mali* Le Baron, which is known as "Hopper Burn." This condition is regarded by some workers as a disease of the "virus" type but it seems to the writer to resemble closely the condition produced by a sucking insect with an unusually toxic salivary secretion. This view is evidently also the opinion of Fenton and Hartzell who put forward the suggestion (p. 410) "that hopper-burn is due to some specific enzymic secretion from the insect itself and is not parasitic in origin."

Further injury by the secretions of these insects to the potato plant includes the collapse and destruction of the epidermal cells over the whole area of the spot; *vide* Plate II, fig. 4.

There is no well-defined stylet sheath surrounding the punctures of *E. auratus* and *Z. pallidifrons* such as are found in the case of certain

aphides. There are however indications of some reaction on the part of the plant with the saliva of the jassid. There is a substance which stains faintly pink with safranin and which forms a thin lining along the passage left by the stylets, *vide* Text-figs. 3, 4. This lining occasionally may be found with an irregular outline exactly following the barbed mandibles of the insect.

The saliva itself stains pink with safranin and a drop may be seen in Plate II, fig. 3, as a somewhat boat-shaped body (*S*) at the apex of the stylets.

This saliva appears to have no power or function of dissolving the middle lamella as is the case with the salivary injections of certain aphides. It has however, as already shown, considerable effect upon the cell contents and chlorophyll of the palisade cells and is altogether more toxic in its effect than is the saliva of aphides.

The damage caused by the feeding of *Myzus persicae* and other aphides apart from any possible infection with virus disease, is more or less confined to the actual puncture but the saliva of the leaf-hopper passes on from the place of puncture and, diffusing through the cell walls, continues to spread till the toxic effect is exhausted.

In the jassids then penetration of the tissue is by pressure. The objective of the stylets is twofold, either the palisade cells or the vascular bundles. There is some mechanical laceration of the cells due to the barbed mandibles. A certain amount of reaction on the part of the cell sap to the saliva is noted. The path of the stylets is wholly intracellular, this is to be expected when the relative sizes of stylets and cells are considered. The toxic effects of the insects' secretions are considerable; the epidermal cells are collapsed and the chlorophyll and contents of the palisade cells are destroyed over a wide area.

HEMIPTERA. HOMOPTERA.

ALEYRODIDAE. *Asterochiton vaporariorum* Westw., "White Flies."

This insect is the well-known greenhouse "white fly" and is a serious pest of tomatoes and other plants. It does not normally attack potatoes unless they are grown for special reasons under glass, when it takes readily to them. In warm weather, however, it has been found by the writer feeding upon potatoes out of doors and may thus be a potential carrier of virus disease.

In this work the white fly was studied mainly in its immobile attached larval form, as being more amenable to fixation and examination *in situ*. The lower leaf surface is always the one selected for attack. Hargreaves (12)

points out that this may be due to one or more of the following reasons: (1) character of the leaf cuticle; (2) presence of stomata; (3) protection from wet; (4) phototropism; (5) position of the anus and character of the excreta. He considers that the reasons for the selection of the under surface mainly consist in the position of the anus and the consequent necessity for the excreta to fall clear of the insect. Nevertheless it should be born in mind that on the under surface the cuticle is certainly

5 A 5 B

Text-fig. 5. 5 A, Stylets of aleyrodes *in situ* in potato leaf, showing penetration to the phloem. Note the curve of the stylets. ×950. 5 B, Penetration of a stoma by the stylets of a larval aleyrodes. ×950.

thinner than on the upper surface and for this reason is more suitable for penetration by the delicate stylets of the young larvae. As regards the stomata Hargreaves is of the opinion that aleyrodes do not penetrate by these. This opinion is not confirmed by the writer's preparations which show that in many cases the stylets, especially those of the smallest larvae, do undoubtedly enter by these openings; see Text-fig. 5 B.

The position of the aleyrodes upon the leaf is always in the neighbourhood of a vein.

The stylets enter the leaf either between two cells or through a leaf stoma. The path is inter-cellular and the objective is usually the phloem vessels of the vascular bundle, although the stylets are often found in the parenchyma alone. Especially is this the case with very small larvae, where possibly the stylets are unable to reach the phloem. Text-fig. 5 A is a drawing of the stylets of a larval aleyrodes *in situ* in a potato leaf. The phloem is the objective in this case. Note the apparent ease, shown by the large bend in the stylets, with which the insect is able to avoid cells and choose an inter-cellular path. Text-fig. 5 B is a drawing of the stylets of a very young larva, which are shown entering the tissue through a leaf stoma and ending in the tissue just below.

There are slight signs of salivary secretion surrounding the stylets which stain faintly with safranin. The writer's preparations of this insect, however, do not show the well-marked stylet tracks ullustrated by Arnaud (2).

The effect of the feeding of aleyrodes upon the tissue of the potato plant is not very evident microscopically, though cells with plasmolysed contents occur along the path of the stylets.

HEMIPTERA. HOMOPTERA.
APHIDIDAE. Species examined: *Myzus persicae* Sulzer.
Macrosiphum solanifolii Ashm.
Myzus circumflexus Buckt.

Myzus persicae Sulzer. The following description of *M. persicae*, apterous viviparous female, is taken from Theobald (20), p. 7:

"Various shades of green, yellow, or pale brownish pink. Antennae slightly shorter than the body, cornicles green, yellow or pink, tips dusky; slightly swollen in middle or just below or above the middle. Cauda pale, about one third length of cornicles. Legs same colour as body, apices of tibiae and the tarsi dark. Proboscis reaches to second coxae."

M. persicae is a common insect upon the sprouts of potato tubers and is also abundant upon the haulms.

A very detailed examination was made of the stylet tracks resulting from the feeding in both the leaf and stem tissue of the potato. Penetration of the leaf takes place as a rule from the lower surface, though occasionally in severe infestation the leaf is punctured from above.

The stylets may enter the leaf tissue in one of two ways, either through one of the stomata, *vide* Plate III, fig. 1, or between two epidermal cells (Plate III, fig. 2). The latter is the more usual point of entry.

The stylets of *M. persicae* follow an inter-cellular course almost exclusively, though individual cells are occasionally punctured *en route.*

In the vascular bundle the phloem is the main objective though the xylem elements are also tapped, the track being sometimes found crossing a xylem vessel diagonally. The tracheides are also tapped, *vide* Text-fig. 6.

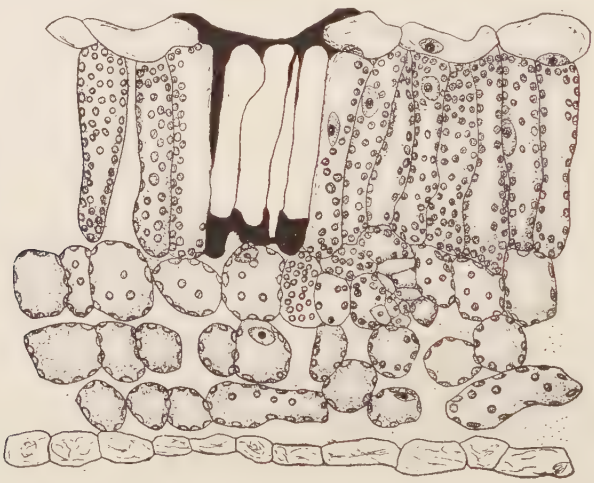
In addition various sections showed that in the case of some punctures, especially those from the upper epidermis, the stylets of *M. persicae* do not invariably penetrate to the vascular bundles, but are confined to the cortex. This is shown in Text-fig 7. Davidson found this also in the case of *Aphis rumicis* and suggests that it may be due to the fact that the insect was killed before it could penetrate further in the tissue. As a rule however the phloem vessels of the vascular bundle are the main objective of this insect.

As regards the effect of the feeding and penetration of *M. persicae* upon the plant tissue, the physical or mechanical damage is too slight to be compared with that caused by the barbed stylets of some of the larger Hemiptera. Those cells actually pierced by the stylets will presumably be destroyed but no laceration of the tissue is apparent. The effect of the salivary secretions however is considerable and appears to be greater than the effect of the secretions of the two other species of aphis studied.

As shown by Davidson and others, the saliva of certain species of aphides has the power of dissolving away the middle lamella between the cells and thus making easy the path of the stylets. This is also demonstrated in the case of *M. persicae.* Adjacent to, though not necessarily in, the path of the stylet track the cell contents are disorganised, the chloroplasts and nuclei being frequently destroyed, *vide* Plate III, fig. 3.

In the epidermal puncture illustrated in Text-fig. 7, three of the palisade cells appear to be quite empty, although the cell walls are still intact (compare leaf-hopper puncture Plate II, fig. 4). The saliva is shown in black deposited on the epidermis and at the base of the palisade cells. The nuclei and contents of the cells in the neighbourhood of the stylet track show a tendency to approach that cell wall nearest to the track (*vide* Text-fig. 6). Marked plasmolysis of the cells and disorganisa-

Text-fig. 6. Epidermal puncture by *M. persicae* in neighbourhood of a vein. Puncture traverses almost the entire diameter of the leaf. Xylem and Phloem tapped. Tracheide filled with saliva. ×800.



Text-fig. 7. Epidermal puncture by *M. persicae*; showing collapse of epidermal cells and destruction of cell contents. ×650.

tion of their contents are caused by the saliva of *M. persicae*. The epidermal cells of the leaf and stem in the area of the puncture are almost invariably destroyed by the excess of saliva, *vide* Plate III, fig. 3.

In Text-fig. 6 may be seen a good example of the tapping of a tracheide and the consequent filling up of the cell with salivary material. It is of interest to compare this drawing with Plate I, fig. 4, a photomicrograph of a puncture by *Lygus pabulinus* where a precisely similar phenomenon is shown.

Plate III, figs. 3, 4, are two photomicrographs of a puncture made by *M. persicae* in the stem of the potato. The track and its inter-cellular course are well shown. This track, as opposed to that in the leaf, shows a characteristic alveolar structure. This can be clearly seen in Fig. 3 and may be due to excess of starch grains embedded in the salivary material. The disorganisation of the cell contents in the neighbourhood of the stylet track is well shown in this figure. Occasionally in the stem punctures, as in the leaf, the excess of saliva is so great that the epidermal cells and those underlying are entirely destroyed giving rise to a cup-like depression on the stem surface. Fig. 4 is a continuation of the stylet track; here the phloem has been reached and tapped. Apparently the aphis has withdrawn its stylets a short distance and re-inserted them to produce the somewhat horse-shoe shaped track illustrated. The phloem can be seen to be completely disorganised and the cells filled with the salivary material. In this section a wound reaction in the phloem can be distinctly seen, the cells dividing abnormally under the stimulus either of the injection or the puncture. This does not agree with the findings of Brandes(4), who investigated the feeding of *Aphis maidis* upon various plant hosts. He says: "There is no apparent wound reaction on the part of the plant, none could be seen in the phloem at any rate."

Macrosiphum solanifolii Ashmead. Theobald(20), p. 5, describes this insect, apterous viviparous female, as follows: "Green or pink, cornicles green darkened at the tips. Cauda pale green or pink, proboscis reaches to second coxae or just beyond. Antennae longer than body, third segment with one to five round sensoria near base. Cornicles about as long as third antennal segment, apices reticulate for about one fifth the length. Cauda nearly half the length of the cornicles. Length 4–4·1 mm."

This aphis was studied *in situ* in leaf and stem tissue of the potato. The stylet track is both intra- and inter-cellular. The comparatively large size of the insect may possibly enable it to penetrate the cell

walls more easily than the foregoing *M. persicae*. The phloem elements were found to be the objective in all the preparations of this aphis.

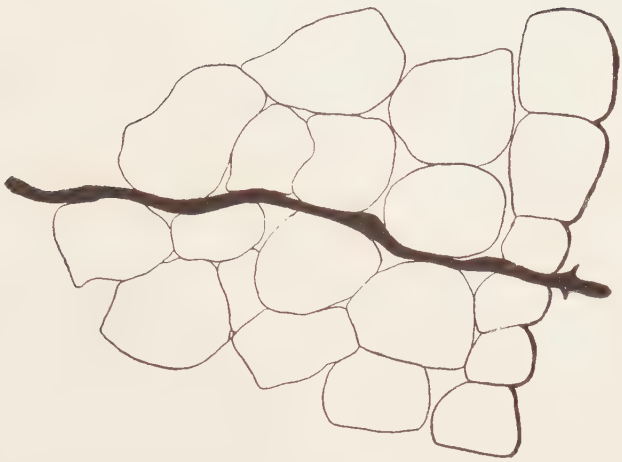
M. solanifolii, then, only appears to differ in its feeding methods from *M. persicae* in the larger number of intra-cellular penetrations noticed and possibly in a slightly less marked reaction of the saliva to the safranin stain.

Myzus circumflexus Buckton. The stylet tracks of the last of the three aphides, *i.e. M. circumflexus*, were then studied. The same fixing and staining methods were used as in the two foregoing species. This aphis is a very common insect upon a great variety of greenhouse plants

Text-fig. 8. Stylet track of *M. circumflexus*; showing branching on approach to phloem. ×950.

and fed readily upon potato leaves. It is characterised by the broad black markings upon the dorsal surface of the abdomen. In this species the track is inter-cellular, the stylets entering the tissue as a rule between two cells. The objective is always the phloem elements of the vascular bundle. It appeared that the stylet tracks of this species though clear enough did not give the strong reaction to safranin characteristic of *M. persicae*.

As the tracks of *M. circumflexus* approach the phloem they are seen to branch in several directions (*vide* Text-fig. 8). This branching of the stylet track in the phloem region is not peculiar to this species but is



Text-fig. 9. Section showing apparent hardening of saliva of aphides, *M. circumflexus*. × 950.

Text-fig. 10. Stylets of *Dactylopius in situ* in potato leaf. Branching of the well-marked stylet track in the phloem is shown. × 800.

probably the case with most aphides and may also be seen in the Coccidae (Text-fig. 10).

In some sections, it was noticed that the saliva stood out from the epidermis in the form of a plug (Text-fig. 9). It was this apparent hardening of the saliva of aphides which gave rise to Busgen's theory that the secretion acted as a supporting structure for the insect's stylets. In its method of tissue penetration, objective of stylets and effect on the cells of the host, *M. circumflexus* does not differ very substantially from the two foregoing species of aphis studied.

From the consideration of the foregoing three species of aphides it would appear that their methods of feeding do not differ to any marked degree. All three appear to favour an inter-cellular method of penetration. At the same time they are able to penetrate intra-cellularly when required as is shown in many instances by *M. solanifolii*. The objective of the stylets in each case is the phloem. The toxic effects of the saliva of *M. persicae* on the cells of the host also appear to be slightly greater than in the other two species. Busgen (5) who studied the feeding of various aphides upon different host plants considers the stylet tracks of these insects fall into three categories as follow:

(*a*) An inter-cellular course to the phloem.

(*b*) An intra-cellular course in the parenchyma.

(*c*) An intra-cellular course to the pholem.

The first of these three tracks is the one applicable to the three aphides herein studied. The second class does not apply here but is discussed in a later section of the paper in relation to other Hemiptera. As regards the third category *M. solanifolii* as already mentioned occasionally follows the intra-cellular course to the phloem but it cannot however be confined to that class.

Though this paper does not deal with the actual composition of the "stylet track" left in the tissue of the host plant by aphides and other sucking insects, the following opinions on the subject are of some interest. Busgen (5) by various chemical tests claims to have proved that the track does not consist of cellulose, nor is it a cuticular substance as it turns yellow with iodide of zinc chlorate. He also says that the track consists purely of animal products because he finds that aphides, when fed upon dead boiled leaves, produce a similar stylet track to that found in normal tissue.

Busgen apparently does not consider that the sap of the plant enters into the composition of the stylet track.

Davidson (6), p. 52, however, summarises his views as follows: "A

well-marked sheath, the stylet sheath, is formed round the piercing organ when it is in the plant tissues, its wall being composed of substances produced by the reaction of the saliva on the cell sap. It probably consists of callose and insoluble calcium pectate, together with depositions of tannin."

In the writer's opinion it would appear as if there must be some reaction on the part of the cell sap to the salivary injections of the insect. In the case of injury to the apple by capsids, and probably in potato injury by the same insect, there is certainly a formation of tannin. Busgen's experiments with the boiled leaves do not seem absolutely conclusive as there might be substances in the tissue, unchanged by boiling, which would still react with the saliva of the insect.

HEMIPTERA. Homoptera.

coccidae. Species studied: *Aspidiotus hederae* Vallot.
Dactylopius longispinus Targ.-Tozz. (H. Britten det.).

Aspidiotus hederae is a true "scale insect," it occurs only under glass and is a common greenhouse pest throughout the British Isles. It feeds upon a variety of plants but the writer was unable to breed it upon the potato plant. It is studied therefore in relation to the tissues of the plant host upon which it was most commonly found, *i.e.* a species of Cordyline.

In both these insects above mentioned and in the Coccidae as a whole, the stylets are very long and hair-like, often far exceeding in length the whole body. It has not yet been satisfactorily explained how these long stylets are forced into the depth of the plant or when in the tissue how they are directed and controlled. In *A. hederae* then entrance to the tissues of the host is made through an epidermal cell and not between two cells. The path of the stylets is exclusively intracellular. This fact is well shown in Plate IV, fig. 1, which is a photomicrograph of the stylets of *A. hederae in situ* in the tissue of the Cordyline. There is a well-marked stylet track which is easily picked out with safranin stain. In the figure the saliva can be seen lining the stylets throughout their length. At one end of the piercing organ in the same illustration, it will be noticed that the stylets have been withdrawn slightly and moved to one side, thus exposing the stylet track.

The contents of the cells along the path of penetration show disorganisation and a shrinking towards the cell wall. This disturbance is very similar in appearance to that produced by the various aphides.

The very long stylets of *Aspidiotus* pass along the tissue in the plane of the leaf surface frequently meeting *en route* the stylets of other individuals upon the same leaf. In consequence of this in studying the species extra care is needed in following the stylet tracks.

There is no evidence to show that this insect taps the vascular bundles. In several sections the stylets were seen passing close to the bundles without penetrating them. Moreover there were found instances of the branching stylet tracks ending in individual cortical cells showing that the contents had been sucked out. Owing to the ease with which the tissue of so resistant a plant as Cordyline is pierced, it is probable that the saliva has a solvent effect upon the cell walls.

This type of penetration would appear to fall into Busgen's second category for aphides, *i.e.* an intra-cellular course through the parenchymatous tissue.

Dactylopius longispinus. The second member of the Coccidae studied was one of the "mealy bugs," *Dactylopius longispinus.* This is not a fixed immobile insect like the foregoing but moves about the plant. It is a very common pest in greenhouses throughout the country. It took readily to the potato plant when grown under glass and bred freely upon it.

Both larval and adult stages in leaf and stem tissue were examined. Elmer(9) claims that this group, *Dactylopius* sp., can carry virus disease.

In this insect, as in *Aspidiotus*, penetration of the tissue is intracellular, even the stylets of newly hatched larvae passing through the epidermal cells.

The path of the stylets of both larvae and adults is invariably intracellular.

There is a well-marked stylet track, the saliva being secreted freely and marking the track of the mouthparts in large irregular drops. In every case, even with newly hatched larvae, the phloem and xylem elements proved to be the objective, the tracks branching very freely in the vascular bundles (Text-fig. 10).

On the leaf the insects are always situated near or along a vein. Sections were cut of a large adult *Dactylopius in situ* upon the stem of a potato plant. The stylets could be seen passing intra-cellularly through the cortex into the vascular bundle.

Some damage is done by this species to the phloem and xylem vessels. The cell walls are badly distorted and their contents disorganised or destroyed. The piercing organ could be traced passing into one of the large thick-walled xylem vessels of the potato stem. A hole larger in

diameter than the stylets could be seen in the wall of the xylem vessel, evidently the saliva of this insect also possesses the power to dissolve the cell walls. The actual disorganisation in the vascular bundle resulting from the feeding of *Dactylopius* appears greater than that produced by the aphides, in so far as the effect spreads in the tissue over a wider area.

In that portion of the piercing organ lying outside the potato stem the saliva surrounded the stylets in large drops which appeared at intervals throughout their length.

In the Coccidae then, as so far studied, there are two types of stylet track, both intra-cellular, but differing in their objective. Both insects appear able to dissolve the cell walls with their salivary secretions. The toxic effects upon the plant of their respective secretions is greater in the case of *Dactylopius*.

Discussion.

It is of interest here to consider the findings of Busgen in regard to the stylet tracks of aphides in relation to the group of Hemiptera as a whole. Busgen finds for aphides three types of track:

(1) Inter-cellularly to the phloem.
(2) Intra-cellularly through the parenchyma, no phloem objective.
(3) Intra-cellularly to the phloem.

It will be seen that it is not possible to confine every species to one particular category.

To the first type, then, belong the three aphides studied and the *Aleyrodes* or "white fly," though one aphis *M. solanifolii* may also be legitimately placed in the third category. To the second type belongs the scale insect *Aspidiotus*, while the "mealy bug" *Dactylopius* and the above-mentioned aphis may be placed in the third category.

The leaf-hoppers and capsid bugs belong to groups two and three as they tap two regions in their feeding. Busgen considers that the explanation of the inter-cellular course lies in the fact that it is the line of least resistance, and that "the insect thereby avoids cells whose contents are unpleasant to it" being an advantage gained by the way.

He explains the intra-cellular course by attributing it to aphides with unusually strong mouthparts, the possession of which enables the insect to ignore the resistance offered by cell walls encountered *en route*. Some of the present writer's findings support these views while others cannot be thus interpreted. It is certainly true that in the larger Hemiptera, capsid bugs and leaf-hoppers, the course of the stylets is almost in-

variably intra-cellular, also *M. solanifolii* which is a large aphis often penetrates the tissue in this manner. On the other hand, as is shown in the section of this paper dealing with the Coccidae, where the stylets, though long are very thin and thread-like, in these insects the course is invariably intra-cellular also. It is difficult to accept the "line of least resistance" theory, as an explanation of inter-cellular penetration when such insects as the Coccidae with their delicate stylets and apparent lack of means of penetrating by pressure should favour intra-cellular penetration. It may be that the type of plant tissue in the host influences the path of the stylets to a certain degree. This might account for the intra-cellular course followed by the coccid through such closely placed cells as are found in Cordyline. It does not however explain why *Dactylopius* should also penetrate intra-cellularly through the loose cortical tissue of the potato plant. Davidson suggests that the importance of the passage of the stylets along the line of the cell walls lies in the support thereby given to the piercing organ. Here again one would expect the coccid to follow an inter-cellular course owing to the delicate nature of the hair-like stylets. It would appear that the explanation lies in the solvent effect of the coccid's saliva which is apparently sufficiently potent to enable the insect to ignore the resistance of the cell walls. This suggestion seems supported by the ease with which *Dactylopius* is able to penetrate the thick xylem vessels of the potato stem and *Aspidiotus* the fibrous tissue of Cordyline.

The inter-cellular course then on this assumption would be followed by those insects unable from their size to penetrate by pressure and whose saliva, though having a solvent effect as in aphides, yet is not potent enough to enable them to go straight for their objective.

What then can be the application of this knowledge of the feeding methods of the Hemiptera to the problem of disease transmission? Supposing the insect to be merely a mechanical carrier of the virus, we may take it that all are potential vectors of the disease. Next comes the question of the inoculation of the plant. The exact part of the tissue tapped may have a considerable bearing upon the question of infection of a healthy plant. There seems little doubt that the phloem elements of the vascular bundle are the best point at which to start an infection as they not only contain substances suitable for the growth of micro-organisms but would facilitate the dispersal of the virus about the plant. It is worthy of mention in this connection that in some virus diseases of the potato, such as leaf-curl, one symptom of the disease, *i.e.* necrosis, is situated in the phloem. This seems to point then, to any insect as a

carrier which taps the phloem of the vascular bundles. Working on this assumption, of the Hemiptera studied the three species of aphis, the aleyrodes, and the mealy bug *Dactylopius* are the insects which are capable of disease transmission. The leaf-hoppers would only inoculate the plant with the virus when they happened to tap the vascular bundles which, as shown in the foregoing study, they do only occasionally. The capsids remain doubtfully incriminated as their objective is necessarily larger and more wide-spread than that of any of the other insects concerned. The only insect which appears to be definitely excluded as a carrier by this method of reasoning is the coccid *Aspidiotus*, whose stylets have an intra-cellular course through the parenchyma and do not tap the vascular bundles.

Another point which must be considered in connection with disease transmission and which may have an important bearing on the question of infection by these insects, is the reaction on the part of the plant to the irritation set up by the stylets and to the toxins in the accompanying secretions. It seems to the writer that the insect which introduces its stylets and saliva to the phloem with the least possible irritation or disorganisation of the plant cells would be the most likely successfully to inoculate the disease. If the internal economy of the plant is seriously upset and if there are indications of wound reaction and secondary cell division round the area of the puncture, the point of infection may tend to be isolated from the normal tissue and inoculation thereby prevented. This latter state of affairs would appear to be well presented by the case of the capsid bug. Here there is a very violent reaction to the salivary secretions and, as shown in Plate I, fig. 5, which illustrates a stem puncture, the tissue is badly disorganised, the cells are killed and the whole is surrounded by a layer of horny substance consisting presumably of the walls of collapsed cells, and a substance formed by reaction to the saliva injected by the insect.

From this point of view the aphides, the aleyrodes, and the mealy bug *Dactylopius* appear to be the most likely insect vectors of virus disease. All tap the phloem and do so without undue disturbance of the cells, with the possible exceptions of the aphis *Myzus persicae* and the coccid *Dactylopius*. In the first there are signs in the phloem of wound reaction and secondary cell division, *vide* Plate III, fig. 4, in the second some disturbance of the vascular bundle. These disturbances however are very slight compared to the disintegration caused by the larger Hemiptera.

Busgen has an interesting theory in regard to the supposed hardening

of excretion of the saliva of aphides which might have a bearing on the question of virus disease transmission by these insects. He suggests, in addition to this hardening being a possible support for the stylets, that it plugs up the entrance to the puncture and thus prevents the entrance of any bacteria. This hardening has been demonstrated in the case of *M. circumflexus* (Text-fig. 9), where the saliva stands out from the puncture hole in the form of a plug. If this theory be correct and if virus diseases are of bacterial origin, it would exclude aphides from acting as vectors of the disease. It is difficult however to determine how much of this hardening of the saliva may not be due to processes of technique.

It remains for the inoculation studies, now proceeding under insect-proof cages with the insects here detailed, to be completed before any confirmation of these theories can be forthcoming.

Summary.

I. The insects studied are selected as being representative of the group HEMIPTERA. They are: Capsidae: *Calocoris bipunctatus* Fab., *Lygus pabulinus* Linn. Typhlocybidae, "Leaf-hoppers": *Eupteryx auratus* Liv., *Zygina pallidifrons* Edw. Aleyrodidae, "White Flies": *Asterochiton vaporariorum* Westw. Aphididae: *Myzus persicae* Sulzer, *Macrosiphum solanifolii* Ashm., *Myzus circumflexus* Buckt. Coccidae: *Aspidiotus hederae* Vallot., *Dactylopius longispinus* Targ.-Tozz.

II. The insects except *Aspidiotus hederae*, are studied chiefly in relation to the potato plant.

III. (*a*) Capsidae. These bugs penetrate the tissue by pressure, the passage of the stylets being assisted by the disintegrating effects of the accompanying saliva. The objective of the beak lies in both cortex and vascular bundles. Both leaf surfaces and stem tissue are punctured. The path through the tissue is intra-cellular. The effect of the saliva upon the cells of the plant host is described in detail.

(*b*) Typhlocybidae. The leaf-hoppers penetrate the plant by pressure. Both leaf surfaces are attacked, the larvae being usually confined to the lower surface. The stem is not punctured as a rule. The objectives of the stylets are the palisade cells and the vascular bundles. The track of the piercing organ is intra-cellular. The effect of the saliva is described, the chlorophyll and cell contents are destroyed over a wide area while the cell walls are left intact. There are some indications of a stylet track.

(*c*) Aleyrodes (White Fly) penetrates inter-cellularly, entrance being made between two epidermal cells, in the case of young larvae often through a leaf stoma. The under surface is always the one attacked. The objective is usually the phloem elements of the vascular bundles, in young larvae the stylets are often confined to the cortex. Damage to the cells of the host was not very evident microscopically, though a few cells showed plasmolysed contents. Very slight indications of a stylet track were visible.

(*d*) Aphides. These insects usually penetrate between two epidermal cells, the track on the whole being inter-cellular. *M. solanifolii* however, a large aphis, often has an intra-cellular stylet track. In each case the phloem was the objective. A well-defined stylet track is formed, most evident in the case of *M. persicae*. Damage by the saliva is largely confined to the actual track of the stylets. Some wound reaction is noted in the phloem caused by *M. persicae*. The under surface of the leaf is usually selected for penetration, the stem is also attacked.

(*e*) Coccidae. Of this group *Aspidiotus* exhibits an intra-cellular path through the parenchyma of the host, in this case a Cordyline. The stylets enter the tissue through an epidermal cell. The phloem is not the objective. There is a well-marked stylet track. Cells in the path of penetration have their contents destroyed. The saliva appears to have the property of dissolving the cell walls.

Dactylopius, a mealy bug, also penetrates intra-cellularly; in this case the phloem and xylem elements are always the objective. On the leaf the insects are situated near or alongside a vein. There is a well-marked stylet track, the saliva being secreted in large irregular drops. Both leaf and stem tissue are attacked. Some disorganisation of the vascular bundles results from the feeding of this insect. That the saliva of *Dactylopius* also dissolves the cell walls is shown by the ease with which it can penetrate large xylem vessels.

A number of photomicrographs illustrating these points are given.

IV. A discussion is appended of the relation of the facts elucidated in the study to the question of the transmission of virus diseases of the potato by such sucking insects.

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EXPLANATION OF PLATES I—IV.

PLATE I.

Fig. 1. Photomicrograph of a section of a potato leaf fixed four hours after puncturing by *Lygus pabulinus*. Note how the proboscis has penetrated the entire thickness of the leaf, and the saliva spreading to right and left. ×800.

Fig. 2. Puncture in potato leaf by *Lygus pabulinus*. Twelve hours after puncturing. Note the shrinkage, and the holes forming in the tissue. ×800.

Fig. 3. Puncture in potato leaf by *Lygus pabulinus*. Twenty-four hours after puncturing. Destruction of the tissue now complete. ×800.

Fig. 4. Section of potato leaf taken outside the area of a puncture by *Lygus pabulinus* showing the spread of the saliva in the tissue. The tracheides can be seen filled with saliva. ×800.

Fig. 5. Section through potato stem punctured by *Calocoris bipunctatus*. Puncture hole surrounded by a layer of saliva and dead cells. Original track of proboscis marked ×. ×800.

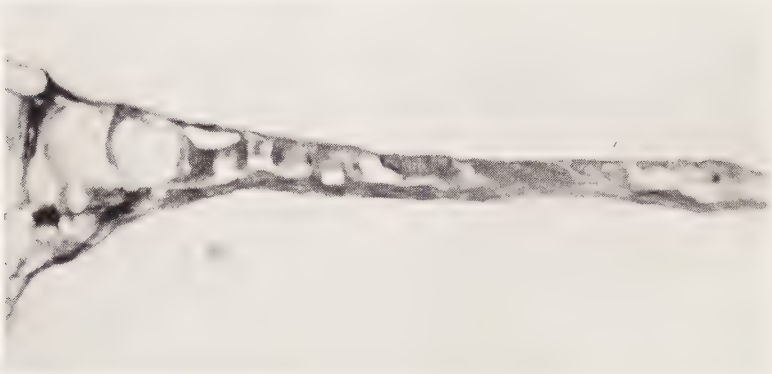
PLATE II.

Fig. 1. Stylets of a leaf-hopper (*Z. pallidifrons*) in the act of penetrating a leaf by pressure. The leaf cuticle and the underlying cell walls are clearly shown breaking under the pressure of the insect's proboscis. The saliva surrounding the stylets appears dark in the photograph. ×800.

Fig. 2. Stylets of a leaf-hopper tapping the palisade cells of the potato leaf. Note the destruction of the chlorophyll. The intra-cellular path of penetration is shown. ×800.

Fig. 3. Stylets of leaf-hopper shown *in situ* in leaf tissue. In this case the objective is a tracheide. The saliva can be seen at the point of the stylets as a somewhat boat-shaped body darkly stained (*S*). ×800.

Fig. 4. Section through a puncture made in a potato leaf by a leaf-hopper. The spot is shown sharply marked off from the normal tissue by the destruction of the chlorophyll. The cell walls can be seen still intact. The epidermal layer is destroyed. ×650.



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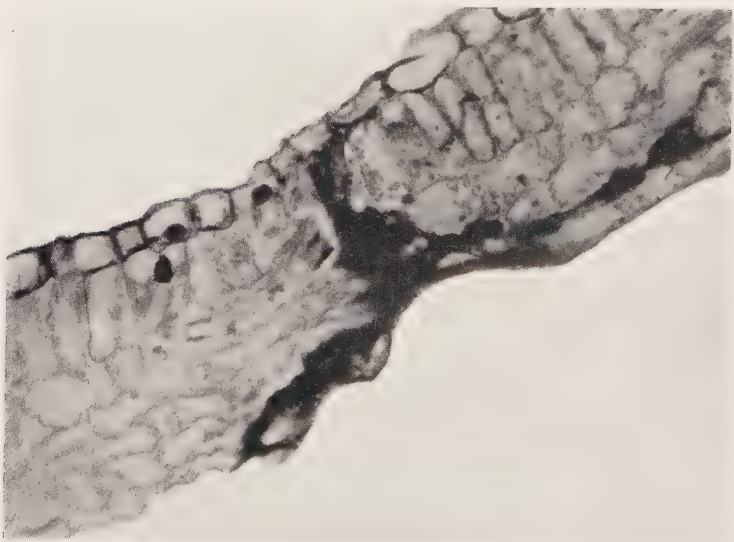

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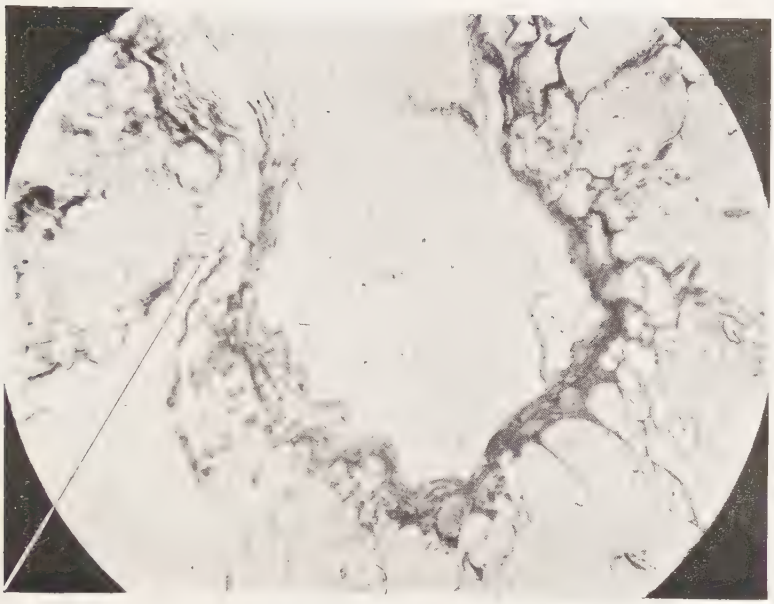

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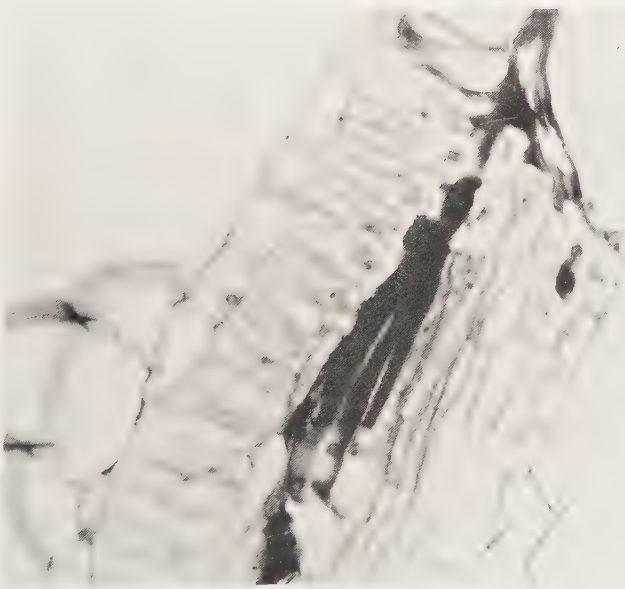

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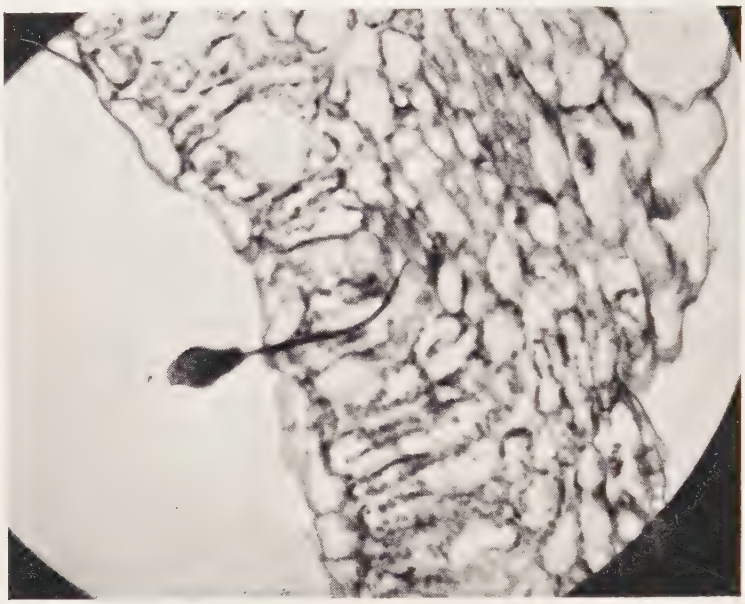
SMITH.—A Comparative Study of the Feeding Methods of certain Hemiptera.



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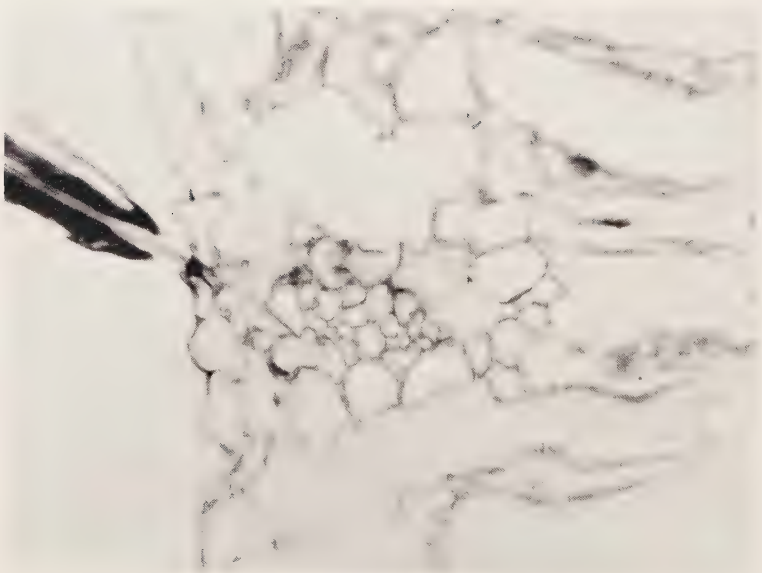

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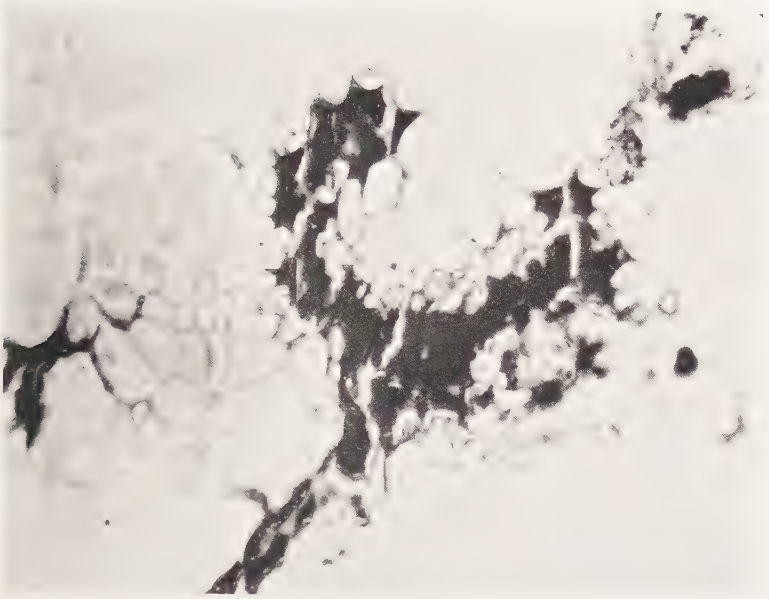

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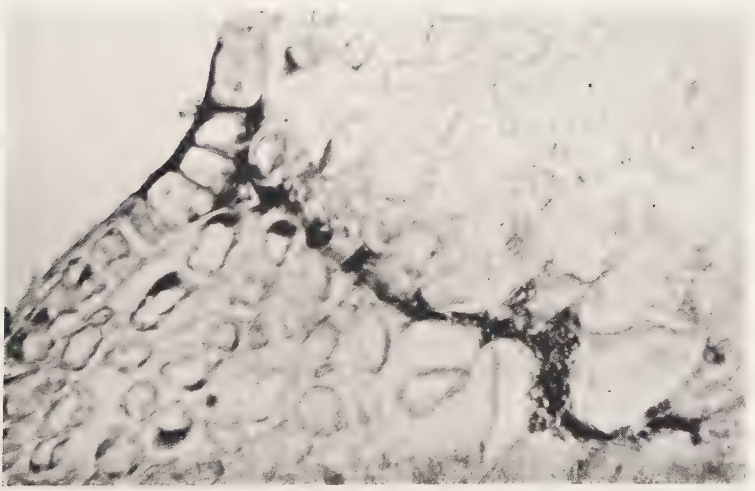

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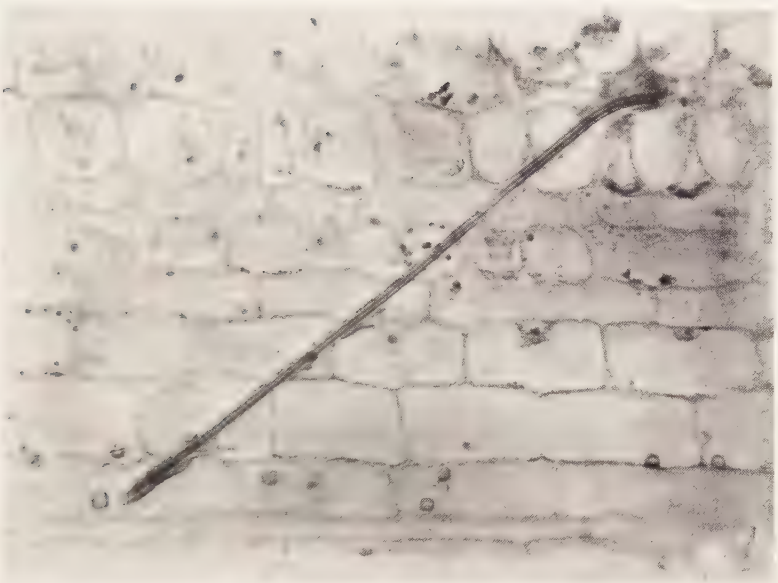


Fig. 1.

SMITH.—A Comparative Study of the Feeding Methods of certain Hemiptera.

PLATE III.

Fig. 1. Photomicrograph of a section of potato leaf, showing the stylets of an aphis, *M. persicae*, in the act of penetrating a stoma. The objective is the phloem which can be seen just below. ×650.

Fig. 2. The stylets of *M. persicae* shown *in situ* in potato leaf. Note how the piercing organ has turned aside to tap the phloem. ×650.

Fig. 3. Photomicrograph of the stylet track of *M. persicae* in the tissue of potato stem. Note the inter-cellular course of penetration. The disintegration of the cell contents near the stylet track is well shown. The cellular appearance of part of the stylet track may be due to excess of starch grains embedded in it. ×800.

Fig. 4. Stylet track of *M. persicae* ending in the phloem. The cells are filled with the salivary material. Some indication of wound reaction may be seen. ×800.

PLATE IV.

Fig. 1. Section of leaf of *Cordyline* showing the stylets of *Aspidiotus in situ*. The intracellular path is well shown. Note the saliva enveloping the stylets on each side, the stylet track exposed where the piercing organ has turned aside, and the plasmolysed cell contents. ×800.

(*Received September 10th*, 1925.)

ON THE NYMPHAL FORM OF THE HARVEST BUG (*TROMBICULA* (*NEOTROMBICULA*) *AUTUMNALIS* SHAW)[1]

By STANLEY HIRST.

(With 2 Text-figures.)

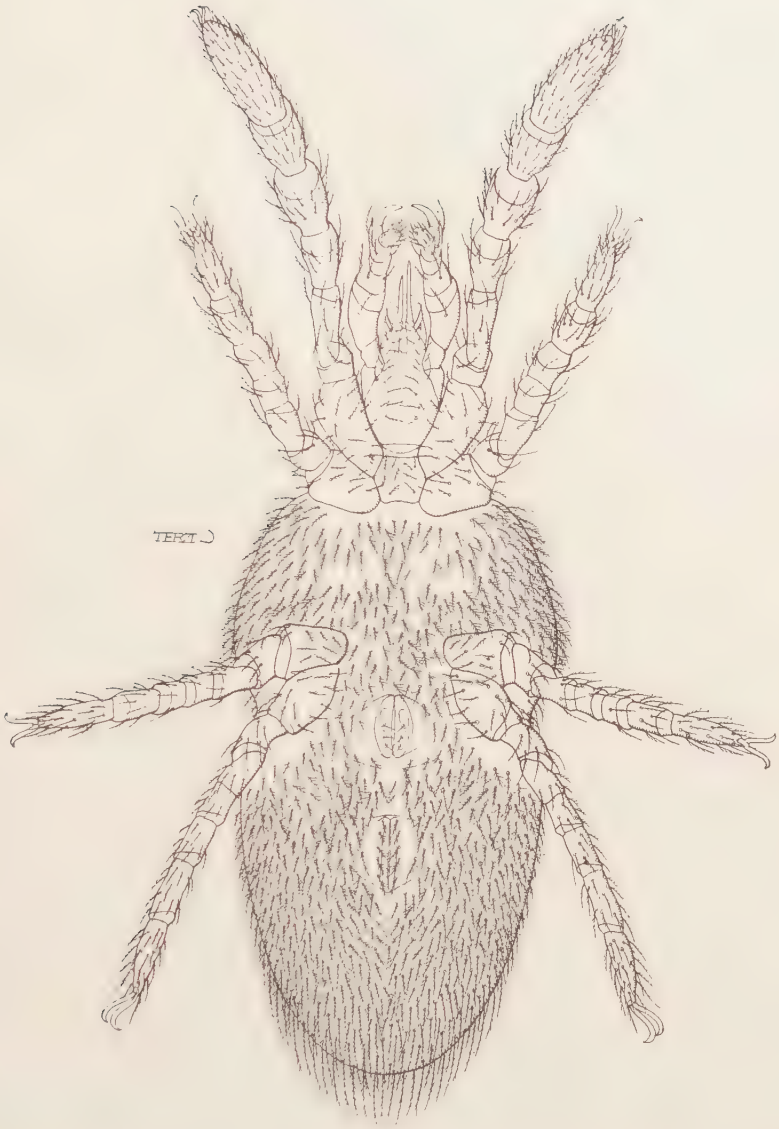
The later stages of the larval mite usually called the Harvest Bug ("*Leptus*" *autumnalis* Shaw) which is so troublesome in many rural districts during the late summer and autumn are still not at all well known. During August of the present year Professor F. V. Theobald of the South-Eastern Agricultural College and Mr C. W. Goode of the National Poultry Institute at Wye sent me some live chickens heavily infested with a larval Trombidiid mite which when mounted and examined under the microscope proved to be "*Leptus*" *autumnalis*. Several hundred specimens were obtained from one of these birds and placed in jars and cells. Nearly all the specimens soon died however probably through too little humidity. A single nymph referable to the genus *Trombicula* appeared in one of the glass cells thirty-three days after the larvae had been placed therein. One or two other larvae also assumed the elongate nymphochrysalis stage which seems to be characteristic of this species of *Trombicula*.

The adult of the Japanese Harvest Bug called "Akamushi" carrier of the Kedani or River Fever (Pseudotyphus?) in Japan is now known to be a species of *Trombicula*. Quite recently Ewing has shown that the common Harvest Bug of North America also belongs to the genus *Trombicula*. It is not very surprising therefore to find that our English Harvest Bug is referable to the same genus. The genus *Trombicula* has not been recorded as occurring in this country but this is no doubt due to the small size of the mites of this genus and probably also to their retiring habits for it seems probable that the later stages live beneath the surface of the ground[2].

Trombicula autumnalis differs from the hitherto described species of

[1] Published by permission of the Trustees of the British Museum.

[2] The Harvest Bug often occurs in the ears of field mice and voles (*Apodemus sylvaticus*, *Microtus glareolus*, etc.), and it seems probable that the adult stage will be found to inhabit the nests of these rodents.



Text-fig. 1. Female nymph of "Harvest Bug" (*Trombicula* (*Neotrombicula*) *autumnalis* Shaw). Ventral view.

the genus in having the body more elongated and also in the differently shaped hairs on the dorsal surface of the cephalothorax, for these reasons I have proposed the new subgeneric name *Neotrombicula* for this species (see *Nature*, October 24th, 1925, p. 609). The name *Leptus* should not be used for *Trombicula autumnalis*. The genus *Leptus* was created by Latreille for the larval mite found on Phalangiids (*L. phalangi*), a species now referred to the family Erythraeidae.

The following is a short systematic description of this bred specimen, *Trombicula* (*Neotrombicula*) *autumnalis* Shaw. (Text-figs. 1 and 2.)

Nymphal form. Colour (when alive) very pale pink. Body rather elongated and not very wide, but with the usual deep constriction

Text-fig. 2. *Trombicula* (*Neotrombicula*) *autumnalis* Shaw. Nymphal form. *a*, Ventral view of palp. *b*. Dorsal view of crista. *c*. Terminal segments of first leg.

characteristic of *Trombicula*. *Eyes* absent. *Crista* typical for the genus *Trombicula*, the pseudostigmal hairs slightly feathered. Hairs on the dorsal surface of the cephalothorax shorter and not shaped as in the described species of *Trombicula*. Hairs at posterior end of body much longer than the others. Hairs on *venter* numerous, short and plumose. *Genital opening*[1] not very long and furnished with two pairs of rather

[1] The female nymphal stage in *Trombicula*, etc. (Trombidiidae) has a distinct genital aperture with two pairs of suckers, instead of the three present in the final (adult) stage. It is interesting to note that there is no sexual opening in the nymphal stages of the Gamasidae and Ixodidae. In Sarcoptidae however it is the (female) nymphal stage that is fertilised by the male, the final female stage being ovigerous.

elongated suckers, also with three pairs of plain hairs. *Anal opening* situated a little posteriorly to the genital one and also rather short; it has a row of short plumose hairs on each edge, similar to those on the rest of the venter. *Palp* with a single strong claw; there are also two much thinner but still fairly stout spines close to the claw. Tarsus of palp thumb-like and furnished with plumose hairs and also a terminal group of five plain unfeathered hairs. There is a distinct hexagonal sternum between the first two pairs of coxae and it is furnished with nine plumose hairs. First coxa with about 14 plumose hairs, second with 9–10, third with 11–12, and fourth with 12 plumose hairs ventrally.

Measurements in mm. Length (including capitulum) ·93, of body only ·72; width of abdomen (behind last leg) ·31; length of tarsus of first leg ·13, its height ·5; length of tibia of first leg ·8.

Note. According to Oudemans the single nymph of the species reared by Bruyant was badly pressed in mounting and the abdomen also damaged so only details of structure can be ascertained. In his important paper on larval Trombidiidae "Die bis jetzt bekannten Larven von Thrombidiidae und Erythraeidae, mit besonderer Berücksichtigung der für den Menschen schädlichen Arten" (*Zool. Jahrb. Syst. Supplement*, vol. 14, 1912), Oudemans states that this nymph bred by Bruyant is referable to *Microtrombidium pusillum* Hermann, but in his later work on Acari occurring in the nests of moles he says he has re-examined the specimen and changes the name to *Microtrombidium autumnalis* regarding it as a distinct species. In spite of some slight discrepancies such as Bruyant's statement that his specimen has eyes, it seems probable that his nymph was also referable to *Trombicula* (*Neotrombicula*) *autumnalis* Shaw.

(*Received October 22nd*, 1925.)

THE FUMIGATION OF TOMATO HOUSES WITH HYDROCYANIC ACID GAS

By E. R. SPEYER and O. OWEN.
(*Experimental and Research Station, Cheshunt, Herts.*)

In recent years, the fumigation of tomato houses with hydrocyanic acid gas for the control of White-fly (*Trialeurodes vaporariorum* Westw.) has become an established practice. Though the pioneer growers knew well the value of this fumigant many years ago, it was in 1920 that Dr Ll. Lloyd, lately entomologist to the Lea Valley Experiment Station at Cheshunt, determined the minimum quantity of sodium cyanide and sulphuric acid to be used in order to produce a maximum mortality of the pest, and, more important still, laid down the conditions under which fumigation could be carried out with minimum injury to the plant (*Annals of Applied Biology*, vol. IX, pp. 1–32, 1922).

The disadvantages of jar cyaniding, as the operation is termed, are as follows:

(1) Separate weighings and measurings are necessary for the sodium cyanide, sulphuric acid and water, and the mixing of the two latter require time and care.

(2) In large blocks of houses, a very considerable number of jars are required.

(3) It is necessary to have one man to every house of a block to drop the cyanide into the acid.

(4) Fumigation is only safe for the plants on still nights, wind currents setting up large concentrations of gas in particular areas of the houses.

Of late, efforts have been made both in the United States, and in Germany, to generate the gas by simpler means as a set off to these disadvantages. The generation of gas from sodium cyanide and sulphuric acid is a quick one, and it is maintained by some that an advantage is gained through the insects being unable to close their stigmata on approach of the gas. We have the gravest doubts as to the validity of this hypothesis, for very weak doses of the gas have an almost immediate effect, temporary though it may be, upon the adult fly.

In our experiments, when the proper amount of gas has been evolved slowly, we have obtained a total mortality for the adult fly, but rather a higher percentage of scale stages have subsequently emerged than with jar cyaniding. On the other hand, we are convinced that the method about to be described involves less risk of damage to plants, and will save very appreciably in the cost of materials and labour as against the practice of jar cyaniding.

The principle we have used is the decomposition of sodium cyanide with sodium bicarbonate. The cyanide in a fairly fine state of division is thoroughly shaken up with powdered sodium bicarbonate, in the proportion of one part by weight of cyanide and three parts by weight of bicarbonate. This mixture is scattered on the paths of the houses at dusk, at the rate of 1 ounce of mixture to every 1000 cubic feet space. The amounts given have been arrived at by a series of experiments, in which gradually increasing quantities of sodium cyanide have been used, always making up the mixture so that there should be sufficient sodium bicarbonate present to decompose the cyanide.

The results of these experiments were as follows:

	Mixture per 1000 c. ft. space		Houses treated		Effect on White fly	
Exp.	Sodium bicarbonate ounce	Sodium cyanide ounce	No.	Total capacity c. ft.	Scales	Adults
(1)	6/7th	1/7th	4	172,000	Not killed	A number recover
(2)	5/6th	1/6th	4	172,000	,,	Killed
(3)	4/5th	1/5th	13	390,000	,,	,,
(4)	3/4th	1/4th	6	252,000	Good proportion killed	,,
(5)	3/4th	1/4th	1	24,000	Killed	,,
(6)	1	1/3rd	1	24,000	,,	,,

In Exps. 1 to 4 there was no injury, though the plants were decidedly on the soft side, and we are doubtful if jar cyaniding could have been resorted to in the case of the plants in Exp. 3. In Exps. 5 and 6 scorching of no very serious nature resulted, due in the latter case to excess of gas evolved and in the former to the plants not having been sufficiently dry at the root at the time of fumigation. In Exp. 5, again, there was much moisture in the air, and the paths were wet, resulting in a persistence of the cyanide vapours throughout the following day.

These experiments show that a mixture of $\frac{3}{4}$ ounce sodium bicarbonate and $\frac{1}{4}$ ounce sodium cyanide per 1000 cubic feet gives the best

results, and that these amounts need not be varied in accordance with the degree of softness of growth of plants or the leakiness or tightness of houses.

In the case of Exp. 4 we had the opportunity of comparing the results with a block of houses fumigated by the jar method, in which the same amount ($\frac{1}{4}$ ounce) of cyanide was used to the 1000 cubic feet.

The percentage of scales killed was slightly in favour of the jar method, but the plants had been slightly scorched in this case.

It is probable in practice that no method of cyaniding will kill all the scales without some injury to the plant, and this has clearly been pointed out by Dr Lloyd (*loc. cit.*). Further the number of adult flies outside the houses is often great and these gain ready access to the houses immediately after fumigation. Provided that dry conditions prevail in the houses, we have found that hardly a trace of cyanide remains in the mixture after 12 hours. We therefore conclude that, economically, the slow generation method is more favourable than the jar method, in respect of risk to the plant and saving in labour. One man can, without the slightest inconvenience, scatter the cyanide-bicarbonate mixture on a large block of houses. As with jar cyaniding, the fumigation should be repeated after a period of 14 to 21 days.

For the convenience of growers, we append detailed directions for the fumigation of tomato houses by the slow process generation of hydrocyanic acid gas in the control of White-fly.

Materials. Dry powdered sodium bicarbonate and high grade sodium cyanide (98 per cent. purity) in a fine state of division, are thoroughly mixed together in proportion of three parts sodium bicarbonate and one part sodium cyanide by weight.

Amount to be used. One ounce of the mixture to every 1000 cubic feet space is distributed upon the paths of the houses, which must be dry. As the generation of cyanide is slow, the operator can treat a considerable number of houses in a block single-handed.

Precautions for the operator. The mixture should not be touched with the hands: when distributing, it should be shaken from a large tin, the operator walking backwards to avoid getting the powder upon his clothing. The ventilators may be opened during distribution, and closed immediately after. The houses should be locked up for the night.

Precautions for the plants. The mixture should be distributed at dusk, water having been withheld from the plants, so that they require moisture at the root. Fumigation on rainy, but not necessarily on windy, nights

is to be avoided. The ventilators should be opened from outside at dawn the next morning, and left open for not less than one hour. The residual sodium carbonate may be left on the paths. Heat may be put on during fumigation if conditions demand it.

In conclusion we wish to thank the growers who so generously placed houses at our disposal for experiment.

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1923 Withycombe, C. L., M.Sc., Ph.D., D.I.C., West Indian Agricultural College, Trinidad, B.W.I.

1921 Woodcock, G. S., Carlton Chemical Works, Glenyall Road, Millwall, London, E. 14.

1914 Wormald, H., D.Sc., A.R.C.Sc., East Malling Research Station, Kent.

1920 Wortley, E. J., F.I.C., M.B.E., F.C.S., Director of Agriculture, Zomba, Nyassaland.

REVIEW

Reproduction in the Rabbit. By JOHN HAMMOND, M.A. (Cantab.), with foreword and chapter on the formation of the *corpus luteum* by F. H. A. MARSHALL, F.R.S. (Biological Monographs and Manuals. General Editors: F. A. E. Crew and D. Ward Cutler.) Edinburgh and London: Oliver and Boyd, 1925. Pp. xxv + 210, Plates XX. 15*s*.

We have been much interested in perusing this, the latest, addition to the Biological Series, and we congratulate the author on a praiseworthy piece of work.

We confess, however, to some doubt as to the exact sphere of usefulness that this presentation will occupy. It is certainly not a manual on the subject of reproduction in the rabbit, though this might be very useful, for it is too discursive and incomplete —there is, for example, no account whatsoever of the process of implantation of the fertilised ovum. It appears to be, rather, a collection of papers loosely connected, and, as they all bear on reproduction in the rabbit, this is taken as the title: it should have been "Certain Aspects of Reproduction in the Rabbit."

Throughout, the author expresses his own views or those he supports in no half-hearted fashion—often on very flimsy grounds—and his arguments are sometimes apparently based on incomplete knowledge: for instance, on page 118 the effect of hyperplasia of the suprarenal cortex in *boys* is not mentioned, yet must be taken into consideration before the effect on girls can be estimated.

The free and easy adoption of the "germinative ferment"—even in inverted commas—gives a sense of unreality whenever it is drawn-upon in explanation. Permissible, perhaps, to Heape, such a term is a sign of weakness in a disciple of the second generation.

We have noted many other sentences and paragraphs throughout the book that detract seriously from its value as a modern scientific work. We give one example: "In birds where the production of yolk calls for great nutritional expenditure only one ovary has survived, so that the highest degree of development in it can be attained." (Page 12.) On what evidence is this statement based? There is, nevertheless, much of the highest value in this volume. The chapter on the effects of lactation, in spite of certain defects, stands out as a most satisfactory piece of research. We hope that this author may be encouraged to continue his investigations in connection with this subject. We should be grateful, too, if Mr Hammond would again examine certain phenomena stated to exist in relation to "pseudo-pregnancy." In doing so he will observe that the frondose appearance of the endometrium, described as specific to this condition, is an appearance commonly seen in domestic rabbits, because they come on heat frequently all the year round. It is a condition identical, in fact, with that seen in women who menstruate too frequently, and which is thought to be due to over-stimulation of the uterine mucosa by ovarian secretion. Sterile coitus, and doe-jumping are not necessary adjuncts to its production in the rabbit.

Mr Hammond's work will stimulate thought and further enquiry, and for this he deserves our best thanks. The volume is very nicely printed and produced, but surely the photomicrographs are quite unworthy of the expense of reproduction.

W. BLAIR BELL.